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A ROLE FOR THE MEDIAL PREOPTIC AREA IN MEDIATING A RESPONSE TO COCAINE

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A ROLE FOR THE MEDIAL PREOPTIC AREA IN MEDIATING A RESPONSE TO COCAINE

by

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Dedication

To my parents for encouraging me to follow my passions and instilling in me a sense of wonder for the natural world.

To my wife for her unwavering love and support.

To my brother for being the only constant in Austin, and for taking the plunge and moving here with me.

To my cousins, by blood and through marriage.

Finally, to my friends for keeping me sane and grounded.

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A role for the medial preoptic area in mediating a response to cocaine

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The salience of natural or drug-associated reward is mediated by phasic dopamine (DA) release in the nucleus accumbens (NAc) arising from DAergic cells in the ventral tegmental area (VTA). Circulating sex steroid hormones can modulate reward associated with drugs of abuse; yet, it still remains unclear which brain regions are responsible for this modulation. The medial preoptic area (mPOA) is a hypothalamic brain area involved in the expression of naturally rewarding behaviors as well as the regulation and reception of circulating sex steroid hormones in female rats. Considering its role in regulating naturally rewarding behaviors, its well-established anatomical connectivity with the VTA, and its responsiveness to circulating sex steroid hormones, the mPOA is an ideal neural node through which hormones could modulate the rewarding facets of drugs of abuse. Here I show that preoptotegmental efferents to the VTA are primarily GABAergic, that they appose putative DAergic cell bodies in the VTA that project to the NAc, and that they are capable of responding to sex steroid hormones and changes in DA release. Furthermore, cocaine influences neural activity in mPOA efferents that project to the VTA. Removal of the mPOA also enhanced cocaine-induced locomotion, Fos-immunoreactivity in the mesolimbic reward system, DA release in the NAc, and augmented conditioned place preference. Together these findings suggest that the mPOA modulates the release of DA in the mesolimbic reward circuitry via inhibitory connections with DA neurons residing in the VTA, and sex steroid hormones, in turn, may act in the mPOA to modulate response to cocaine.

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Chapter 1: Background

Use and abuse of illicit drugs is a worldwide problem. In the United States alone, an estimated 24.6 million Americans above the age of 12 used illicit drugs in 2013. These data also indicate that rates of drug abuse are at their highest since the administration of the current version of the SAMSHA survey began in 2002. Of the illegal drugs, cocaine has one of the highest rates of initiates (>600,000) and it is the second most used illicit drug in the United States (~1.5 million people). The majority of initiates continue to abuse cocaine and many of them become dependent on the drug. Historically, the overwhelming majority of cocaine users have been males, and studies of cocaine abuse, dependence, and treatment have consequently been predominantly male-centric. Yet, over the last few decades the gender gap has narrowed as females abuse cocaine at elevated rates, albeit at about half the rate of men (0.4% vs. 0.8% of the U.S. population; Greenfield, Manwani, & Nargiso, 2003; Substance Abuse and Mental Health Services Administration, 2011). A multitude of studies have found evidence of sex differences in the subjective effects of cocaine, the path to dependence, and the psychosocial problems and health issues related to cocaine use (see Lynch, Roth, & Carroll, 2002 for a comprehensive review). This suggests that there are basic neurophysiological differences between the sexes that influence cocaine use and abuse. There is a need to further explore the neurological underpinnings of cocaine-induced reward in females to better understand the road to addiction. This will not only further our basic understanding of inherent sex differences in reward-seeking behavior, but it will also help guide potential therapies in preclinical and clinical settings.

1.1. COCAINE ABUSE IN FEMALES

There are clear psychosocial differences in cocaine use and abuse between men and women, which underscores the need for an in-depth examination of this gender disparity. Despite the epidemiological data showing that men abuse cocaine at twice the rate of women, women use cocaine at comparable rates given the same opportunity to gain access to the drug (Van Etten, Neumark, & Anthony, 1999). Furthermore, women have a shorter latency from use to dependence than men (Haas & Peters, 2000; McCance-Katz, Carroll, & Rounsaville, 1999; Westermeyer & Boedicker, 2000), they begin abusing at a younger age (Griffin, Weiss, Mirin, & Lange, 1989), and have shorter abstinence periods between bouts of use (Kosten et al., 1996).

Researchers have also found gender differences in both the health consequences of cocaine abuse and treatment outcomes. For example, women who abuse cocaine have more social problems and complications with family dynamics (Weiss, Martinez-Raga, Griffin, Greenfield, & Hufford, 1997). This may be related to increased incidence of premorbid depression and comorbidity with mental disorders in women who use cocaine (Griffin et al., 1989; McCance-Katz et al., 1999). Additionally, crack use in women results in emergency room visits more often than in men (Dudish & Hatsukami, 1996), but this may be due, in part, to women's propensity to use the medical system at higher rates than men (Blume, 1990). The same investigators also noted that women who abuse cocaine are more likely to smoke cigarettes, further compounding potential health issues associated with cocaine use.

There also exist sex differences in response to treatment for cocaine abuse. Women tend to be younger at the age of first treatment (Griffin et al., 1989), but also drop out of treatment programs more quickly (Siqueland et al., 2002). Fortunately, women have a propensity to abstain from cocaine use for longer time after completion of

treatment (Weiss et al., 1997). Gender-specific substance abuse treatment services might improve outcomes for both men and women. Although implementation of such treatment services in clinics has been slow, they have been successful when used (see Green, 2006 for review). Given these inherent sex differences in outcomes of treatment for cocaine addiction and health effects of cocaine use, further studies of cocaine-mediated reward mechanisms in women become increasingly clear.

1.1.1. Clinical findings

Most studies find no evidence for sex differences in pharmacokinetics, pharmacodynamics, or physiological effects (e.g., tachycardia) of cocaine regardless of route of administration (Collins, Evans, Foltin, & Haney, 2007; Evans & Foltin, 2006; Evans, Haney, Fischman, & Foltin, 1999; Mendelson et al., 1999; but see Lukas et al., 1996). Yet, several studies indicate that women perceive physiological effects of cocaine differently. In particular, they feel a greater sense of nervousness after nasal insufflation (Kosten et al., 1996), or oral administration (Singha, Mccance-Katz, Petrakis, Kosten, & Oliveto, 2000) of cocaine. They exhibit greater latencies from time of administration to initiation of the subjective effects of cocaine and lower levels of dysphoria and euphoria with intranasal cocaine use (Lukas et al., 1996). Furthermore, Sofuoglu and colleagues (1999) found that women had lower perceived tachycardia and paranoia than men. Contrary to what one might hypothesize, these attenuated perceptions do not mitigate women's responses to relapse-related stimuli. Notably, women have increased cue reactivity (Robbins, Ehrman, Childress, & O'Brien, 1999) and greater craving during abstinence (Elman, Karlsgodt, & Gastfriend, 2001).

These findings point to sexually divergent responses to cocaine, but it is important to consider some caveats that are almost always associated with studies of drug use.

Human studies, often, have several confounding variables inherent to the experiment, because drug abusers are often polydrug users and differences in prior drug use between the sexes, which can skew experimental results, are difficult to control for (Gouzoulis-Mayfrank & Daumann, 2006; Schensul, Convey, & Burkholder, 2005). The use of animal models to study sex differences in drug use and reward help overcome many of these challenges.

1.1.2. Preclinical findings

1.1.2.1. Pharmacokinetics

Several studies suggest that differences in sex-specific behavioral responses to cocaine are driven by neurobiological rather than by sexually divergent cocaine pharmacokinetics. There are many biological factors, such as concentrations of enzymes responsible for the degradation of cocaine in the brain and liver that play a role in determining cocaine pharmacokinetics, and these factors are likely to both differ between species and be affected by the hormonal status of the female. Unlike the human data, the preclinical studies are divided with regards to sex differences in pharmacokinetics. The majority of rodent studies report robust sex differences in cocaine metabolite levels in the brain and plasma, yet not levels of cocaine itself, following acute administration (see Quiñones-Jenab, 2006 for review); studies differ on which metabolites in the brain are different between the sexes (Bowman et al., 1999; Festa & Quiñones-Jenab, 2004; Festa et al., 2004; Niyomchai, Jenab, Festa, Akhavan, & Quiñones-Jenab, 2006). On the contrary, non-human primate studies have demonstrated that there are little to no sex differences in cocaine pharmacokinetics (Mello, Bowen, & Mendelson, 2002; Mello, Sarnyai, Mendelson, Drieze, & Kelly, 1993; Mendelson et al., 1999), which is

comparable to the aforementioned studies using human participants.

1.1.2.2. Phases of cocaine dependence

In preclinical models, acquisition of cocaine-seeking behavior is usually defined as the frequency of self-administered intravenous drug infusions over the first few days of the experiment or as the establishment of conditioned place preference. Acquisition can be used to assess the subjective and motivational effects of a drug (Bardo & Bevins, 2000). Using these operant conditioning paradigms, research has shown that there are definitive sex differences in the acquisition phase of cocaine-seeking behavior. A higher percentage of female rats successfully acquire cocaine self-administration compared to males, and they do so in a shorter length of time (Jackson, Robinson, & Becker, 2006; Kerstetter, Aguilar, Parrish, & Kippin, 2008; Lynch & Carroll, 1999; Lynch, Roth, Mickelberg, & Carroll, 2001; Lynch, 2008). Moreover, longer periods of access to cocaine during self-administration procedures enhance this sex difference (Roth & Carroll, 2004). Female rats (Lynch, 2008; Roberts, Bennett, & Vickers, 1989; see Haney et al., 1995 for opposing results) and monkeys (Mello, Knudson, & Mendelson, 2007) also reach higher self-administration breakpoints under a progressive ratio schedule. Finally, a greater percentage of food-restricted female rats choose cocaine infusions over food in a concurrent reinforcement paradigm as compared to males (Kerstetter et al., 2012). Studies utilizing conditioned place preference (CPP) showed similar results. (Russo and colleagues (2003) found that female rats exhibited more entrances into the cocaine side compared to male rats, but did not show a difference in overall CPP scores (difference in time spent in cocaine-paired chamber between pretest and post-test). The same lab found that female rats acquire CPP at lower doses and in a shorter time period than males (Russo, Festa, et al., 2003).

While there are ample data on behavioral differences between the sexes during cocaine acquisition, less research has been done on the maintenance, extinction and reinstatement phases of cocaine use. Females display an exaggerated dysregulation of self-administered cocaine infusions over several sessions compared to males (Lynch et al., 2002). There are contradictory reports on the quantity of cocaine that females self-administer compared to males during the maintenance phase (Jackson et al., 2006; Lynch, Arizzi, & Carroll, 2000). However, this may be a function of the amount of time that the animals are allotted access to cocaine, as a longer access period leads to increased rates of consumption by females compared to males (Roth & Carroll, 2004). Once maintenance is established, researchers utilize abstinence, extinction, and reinstatement protocols as a representation of withdrawal and relapse in humans. Overall, females exhibit a greater resilience to extinction of cocaine-seeking behaviors (Kerstetter et al., 2008; Perry, Nelson, & Carroll, 2008). For example, female rats, whether or not they are given priming injections of cocaine, press the previously active lever during the extinction phase at higher rates, particularly after 60 and 180 days of withdrawal (Kerstetter et al., 2008). Much like the initial phases of cocaine abuse, the withdrawal and reinstatement phases in females are also more pronounced than in males.

1.2. APPROPRIATING THE MESOLIMBIC REWARD SYSTEM

From an evolutionary perspective, it is difficult to see how the use of most drugs of abuse could be an advantageous behavior, as they do not provide adaptive benefits, reproductive advantages, or increase evolutionary fitness (Wise, 1982, 1996). If anything, addiction to psychostimulants is maladaptive considering the health consequences (Viscarello, Ferguson, Nores, & Hobbins, 1992; Washton & Gold, 1984) and increase in detrimental risk-taking behaviors (Edlin et al., 1994; Tapert, Aarons, Sedlar, & Brown,

2001). Drugs of addiction, dopamimetics in particular, act on mechanisms that are responsible for integrating and processing naturally rewarding stimuli.

1.2.1. Reward and the mesolimbic system

1.2.1.1. Overview of the mesolimbic dopamine system

The mesolimbic dopamine (DA) system is the canonical neuroanatomical system that regulates natural and drug-mediated reinforcement. DA-producing cell bodies in the mesencephalic ventral tegmental area (VTA) have complex interactions with a multitude of midbrain and forebrain regions (Ikemoto, 2007). In particular, researchers have focused on projections from the VTA to the ventral striatum as a crucial component of the circuitry thought to be responsible for reinforcement, and reward processing and prediction. The VTA can be divided into four distinct regions based on the types of neurons present and the brain areas targeted by projections. Both the parabrachial pigmented nucleus (PBP) and paranigral nucleus are rich in DA-producing neurons. The parafasciculus retroflexus area (PFR) at the rostral extreme of the VTA and the tail of the VTA (VTT; Ikemoto, 2007), now more commonly referred to as the rostromedial tegmental nucleus (RMTg; Jhou et al., 2013; Maroteaux & Mameli, 2012; Matsui, Jarvie, Robinson, Hentges, & Williams, 2014) at the caudal extreme of the VTA both contain mostly GABAergic neurons. The PN, PBP and RMTg are of particular relevance here, since the PFR does not project to the ventral striatum or influence tegmentostriatal connections (Ikemoto, 2007).

The GABAergic cells of the RMTg project mainly onto DAergic cells in the PBP, PN, and the substantia nigra pars compacta (SNc), providing tonic inhibitory input (Hong, 2011). This inhibition controls DA release by the PN and PBP, which each send projections to different areas of the limbic system (Ikemoto, 2007). A single DAergic cell

from these areas can have vast axonal arborization in the forebrain, usually within the same region (Matsuda et al., 2009). Neuroanatomically, the PN is the most medial region, extending from the ventral midline, whereas the PBP is just lateral to the PN and extends to the dorsal aspects of the SNc. Interestingly, the terminals of these DAergic projections in the ventral striatum follow the same mediolateral gradient that is seen in their respective VTA somata. In other words, the PN and the more medial aspects of the PBP project mainly to the ventromedial striatum, whereas the lateral portions of the PBP project to the ventrolateral striatum (Ikemoto, 2007). This distinction becomes important when examining the functional roles of the ventral striatum.

Accordingly, the ventral striatum is also subdivided into two distinct neuroanatomical regions: the olfactory tubercle (OT) and the nucleus accumbens (NAc). Both regions are multisensory processing centers and integrate a variety of external stimuli and internal cues (Carlezon & Thomas, 2009). The NAc is further divided into core (NAcC) and shell (NAcS) regions. Likewise, these are cytoarchitecturally (Dumitriu et al., 2012), functionally (Bassareo & Di Chiara, 1999; Di Chiara, 2002; Ito, Robbins, & Everitt, 2004; Sokolowski, Conlan, & Salamone, 1998) and histochemically (Lu, Ghasemzadeh, & Kalivas, 1998; Zahm, 2000) distinct regions that receive differential input from the VTA (Ikemoto, 2007). Neurons in the medial NAcS receive the majority of the their DAergic input from PN and medial PBP, whereas neurons in the lateral NAcS and the NAcC receive the majority of their innervation from the lateral PBP and nigrostriatal system (Deutch & Cameron, 1992; Ikemoto, 2007).

The OT and both subregions of the NAc are composed mainly (>90%) of GABAergic medium spiny neurons (MSN) that provide tonic inhibitory input to cells in the ventral pallidum - an area that serves as the limbic-motor interface (Smith, Tindell, Aldridge, & Berridge, 2010). Phenotypically, MSNs are generally classified as either D1-

type neurons or D2-type neurons (Gerfen et al., 1990). D1-type cells express D_1-like DA receptors, produce dynorphin and substance P, are responsive to low levels of tonic DA from the VTA, and typically project to the substantia nigra. D2-type cells express D_2-like DA receptors and produce enkephalin, are activated by phasic release of DA from the VTA (Albin, Young, & Penney, 1989; Surmeier, Ding, Day, Wang, & Shen, 2007) and project to the pallidum (Heimer, Zahm, Churchill, Kalivas, & Wohltmann, 1991). This phasic inhibition of the medial NAcS disinhibits the VP, allowing for a locomotor response (Smith et al., 2010).

1.2.1.2. Processing reward

Homologues of the mammalian mesolimbic DA system are present throughout vertebrate phylogeny (O'Connell & Hofmann, 2011). This neural system is responsible for assessing salient environmental stimuli, integrating it with the internal state of the animal, and producing an appropriate behavioral response (Deco & Rolls, 2005; Wickens, Budd, Hyland, & Arbuthnott, 2007). As discussed above, the mesolimbic DA system receives input from a variety of cortical and subcortical structures that are responsible for interpreting or relaying external stimuli (Grace, Floresco, Goto, & Lodge, 2007; O'Donnell & Grace, 1995). Moreover, it receives input from several diencephalic nodes (e.g., bed nucleus of the stria terminalis) that regulate and integrate hormonal signals, which relay information about the internal state (Fahrbach, Morrell, & Pfaff, 1986; Ikemoto & Bonci, 2014; Jalabert, Aston-Jones, Herzog, Manzoni, & Georges, 2009; Kudo et al., 2012). It is the VTA that then relays information to the NAc via DA signaling about the salience of these stimuli, which help the animal to assess the adaptive value, attach valence, and produce an appropriate behavioral output (Di Chiara et al., 2004; Hart, Rutledge, Glimcher, & Phillips, 2014; Saddoris, Sugam, Cacciapaglia, &

Carelli, 2014; Sokolowski et al., 1998).

Exactly how the DA signal to the NAc assists in assessing the adaptive value and attaching valence is unknown. There are a number of hypotheses that attempt to explain this phenomenon. Of these, only the most well established hypotheses will be discussed here (see Ikemoto, 2007 for a comprehensive review): the *anhedonia theory* of DA-mediated reward; the *incentive salience/formation theory*; and the *reward prediction-error theory*. All of these theories stem from preliminary observations that the mesolimbic DA system influences motivation to obtain reinforcers. In particular, it was Ungerstedt's (1971) account that was one of the first to show that selective lesions of DA fibers projecting to the striatum disrupt motivation to obtain food.

This and similar observations that neuroleptics block reinforcement ((i.e., anhedonia; Fouriezos, Hansson, & Wise, 1978; Yokel & Wise, 1976) stemmed the anhedonia hypothesis, which is an axiom in the DA hypothesis of reward (Fibiger, 1978; Wise, Spindler, Dewit, & Gerber, 1978; Wise, 1978, 1982). The anhedonia theory posits that stable tonic DA release in the striatum is responsible for normal functioning, but that a stimulus that evokes a phasic or sustained increase in DAergic tone reinforce learned reward-seeking behaviors (Wise, 1982). For example, the subjective hedonia experienced by the organism as a function of stimulus acquisition correlates with the increase in DA (Koob & Le Moal, 2001; Volkow, Fowler, & Wang, 1999). However, critics of this hypothesis counter that the subjective experience elicited by a reinforcing stimulus cannot be assessed by behavior alone (Ikemoto, 2007). Moreover, Cannon and Palmiter (2003) reported that genetically altered mice lacking the ability to produce DA preferred sucrose over water and non-caloric, sweet saccharin, suggesting that DA is not necessary for motivation to obtain natural rewards.

Following the DA hypothesis of reward, the incentive salience/formation theory

of DA action was first proposed by Robinson and Berridge (*incentive salience*; 1993, 2003), and modified by Ikemoto and Panksepp (*incentive formation*; 1999). The first manifestation of this hypothesis posited that striatal DA "transforms the brain's neural representations of conditioned stimuli, converting an event or stimulus from a neutral 'cold' representation (mere information) into an attractive and 'wanted' incentive that can 'grab attention'" (p. 5; Robinson & Berridge, 1993). However, this hypothesis only accounts for the wanting (appetitive) aspect and not the liking (consummatory) aspect of goal-directed behavior, which is also correlated with striatal DA release. The incentive formation hypothesis similarly argues that appetitive motivation is reliant on striatal DA (Berridge & Robinson, 1998; Blackburn, Pfaus, & Phillips, 1992; Ikemoto & Panksepp, 1996), but Ikemoto and Panksepp (1999) further hypothesized that increased DA during "liking" attaches a positive valence to the stimulus, whereas decreased or stable DA output during this phase reduces the incentive value. A report by Stuber and colleagues (2005) using *in vivo* fast scanning cyclic voltammetry to assess rapid, phasic DA release demonstrated that during consecutive extinction trials striatal DA response decreases steadily. In sum, DA release is, at least in part, responsible for appetitive motivation and is responsible for re-evaluating the valence of the stimuli during the consummatory phase.

A final neurophysiological phenomenon related to DA release, which is not included in the incentive salience/formation hypotheses, is known as reward prediction error. This phenomenon, first discovered by Schultz (1998, 2000, 2002), describes the transfer of firing of DA neurons in the VTA from an acute reinforcer to a predictive conditioned stimulus after repeated pairings of the two. Importantly, although the same neurons will depolarize when presented with reinforcer-associated cue, they will hyperpolarize if the unconditioned stimulus is not delivered; thus, exhibiting ability to

code an error in response. This theory, however, does not completely describe the role of VTA DA neurons. For example, the tegmental and nigral DAergic neurons examined may project to different striatal targets and the downstream consequences of these proximal electrophysiological responses remain unknown. Notwithstanding, the results of these experiments add to our overall understanding of the mesolimbic DA system function and may inform the aforementioned hypotheses.

Overall, motivation, reinforcement, and reward processing are regulated by complicated neurochemical responses in a variety of neural networks, and active research on this topic is still ongoing (reviewed in Baik, 2013; Carlezon & Thomas, 2009; Covey, Roitman, & Garris, 2014; Holmes & Fam, 2013; Ikemoto & Bonci, 2014; Ikemoto, 2007; Koob & Volkow, 2010; Volkow, Wang, & Baler, 2012; Wise, 2009, 2004, 2008). Further complicating our understanding, the neuroanatomy and neurochemistry involved in these processes overlap with many circuits and molecular mechanisms associated with learning and memory (Lüscher & Malenka, 2011). Although the role of DA in incentive learning and reward processing is not fully understood, it is being redefined continuously with novel techniques and further pharmacological studies.

1.2.2. Natural versus cocaine-associated reward

Both natural rewards (e.g. sex) and cocaine consumption in humans, along with a wide variety of other drugs of addiction, share a similar, euphoric "rush" (Levin, 2014; Seecof & Tennant, 1986). Moreover, anticipation and consumption of cocaine in rats are associated with analogous unconditioned behavioral responses to natural rewards; for example, the emission of reward-associated ultrasonic vocalizations (Barker et al., 2010; Browning et al., 2011; Ma, Maier, Ahrens, Schallert, & Duvauchelle, 2010). Natural rewards, like drugs of addiction (Robinson, Becker, & Presty, 1982; Robinson & Becker,

1982; Robinson & Berridge, 2008; Segal & Mandell, 1974) can sensitize the DA response in the mesolimbic system (Kohlert & Meisel, 1999), and their reinforcing properties can be measured by operant responding (Beach & Jordan, 1956; Caggiula & Hoebel, 1966; Crawford, Holloway, & Domjan, 1993; Everitt & Stacey, 1987) and conditioned place preference (Martínez & Paredes, 2001; Raúl G Paredes & Vazquez, 1999; Tzschentke, 2007). There is also a cross-sensitization that occurs with psychostimulants and natural reinforcers. Notably, exposure to amphetamine in sexually naïve female rats increases appetitive sexual behaviors when they are exposed to a male for the first time (Afonso, Mueller, Stewart, & Pfaus, 2009). These commonalities in and cross-sensitization of behavioral responses are likely due to similar underlying neurochemical mechanisms. Yet, while natural reinforcers also influence a variety of neural circuits and neurochemical cascades outside of the mesolimbic system, cocaine exerts the majority of its reinforcing influence by acting specifically at DAergic synapses in the NAc (Bowman & Richmond, 1996; Carelli & Deadwyler, 1994, 1996; Carelli & Deadwyler, 1997; Chang, Janak, Woodward, & Carolina, 1998; Chang, Sawyer, Lee, & Woodward, 1994; Peoples & West, 1996; Peoples, Gee, Bibi, & West, 1998).

Pharmacological agents, including cocaine, have helped delineate the functional neuroanatomy and microcircuits of the mesolimbic DA system. The main action of cocaine is as a monoamine transporter inhibitor (Kuhar, Ritz, & Boja, 1991; Ritz, Lamb, Goldberg, & Kuhar, 1987; Wilcox, Paul, & Woolverton, 1999). Specifically, these transporters are membrane-associated proteins that remove the neurotransmitters from the synaptic cleft and transport them into the cytosol of the cell, thus controlling the monoaminergic stimulation of the post-synaptic cell. In rats, the ratio of cocaine-mediated monoamine reuptake inhibition in the brain is serotonin-to-dopamine at 2:3 and serotonin-to-norepinephrine at 2:5 (Rothman et al., 2001). Yet, the most studied action of

cocaine is as a DA transporter (DAT) inhibitor in mesolimbic synapses. This may stem from the current popularity of the DA hypotheses of reinforcement (Spanagel & Weiss, 1999), as discussed above. Though cocaine inhibits norepinephrine clearance more strongly than DA clearance, the norepinephrine hypothesis of reward (Stein, 1968) fell out of favor by the mid-1970's as pharmacological and anatomical findings contradicting this hypothesis accumulated (see Wise, 2008 for review).

1.2.2.1. Ventral tegmental area

The VTA provides strong DAergic input to the NAc, and is directly influenced by reward-associated stimuli. Lesions of the VTA generally attenuate naturally rewarding behaviors. Ablation of the VTA significantly impairs receptive sexual behavior in female rats (Herndon, 1976). The expression of maternal behaviors, similar to sexual behaviors, is diminished after electrolytic or 6-hydroxydopamine (6-OHDA) lesions of the VTA (Hansen, Harthon, Wallin, Lofberg, & Svensson, 1991; Numan & Smith, 1984). Likewise, VTA lesions disrupt operant cocaine seeking behaviors (Roberts & Koob, 1982). Pharmacological inhibition of VTA DA neurons attenuates operant responding for sucrose and food (Cacciapaglia, Wightman, & Carelli, 2011; Ranaldi et al., 2011) disrupts maternal behaviors and place preference for pup-paired cues (Numan, Stolzenberg, Dellevigne, Correnti, & Numan, 2009; Seip & Morrell, 2009), impairs cocaine-associated conditioned approach behavior (Ikemoto and Panksepp, 1996), and prevents cocaine-primed reinstatement (McFarland & Kalivas, 2001). Overall, acquisition of responding for or presentation of cues associated with natural rewards increase activity of the VTA (Balfour, Yu, & Coolen, 2004; Hernández-González, Navarro-Meza, Prieto-Beracoechea, & Guevara, 2005; Kest, Cruz, Chen, Galaj, & Ranaldi, 2012; Parada, Chamas, Censi, Coria-Avila, & Pfaus, 2010; Pfaus & Heeb,

1997), but cocaine's action on the VTA is not as clear. Electrophysiological studies have shown that acute cocaine inhibits VTA firing (Brodie & Dunwiddie, 1990; Bunney, Appel, & Brodie, 2001). Immunohistochemical analyses of the protein product of the immediate early gene, *c-fos*, which is indicative of neural activity in response to a stimulus, support the electrophysiological data, but demonstrate that activity in the VTA increases after chronic treatment with cocaine (Zahm et al., 2010) and in response to conditioned cocaine-associated cues (Kufahl et al., 2009). Yet, a study in human subjects using fMRI reported that acute cocaine increases activity in the VTA (Kufahl et al., 2005). Regardless of this discrepancy, optogenetic methods have demonstrated that the VTA directly mediates the reinforcing and rewarding aspects of cocaine and natural stimuli (reviewed in Stuber, Britt, & Bonci, 2012).

1.2.2.2. Nucleus accumbens

Activity in the VTA, which is mediated by cocaine or natural incentives, then translates into neurochemical and electrophysiological responses in the NAc. Similar to the VTA, lesion studies demonstrated that the NAc is necessary for the rewarding and reinforcing aspects of cocaine (Everitt, 1990; Ito et al., 2004; Pettit, Ettenberg, Bloom, & Koob, 1984; Roberts, Koob, Klonoff, & Fibiger, 1980; Roberts & Koob, 1982), maternal behavior (Hansen et al., 1991; Hansen, 1994; Lee, Clancy, & Fleming, 1999; Li & Fleming, 2003; Numan, Numan, Schwarz, et al., 2005), sexual behavior (Hansen et al., 1991; Hull & Rodriguez-Manzo, 2009; Y.-C. Liu, Sachs, & Salamone, 1998), and feeding behavior (Kelley & Stinus, 1985; Koob, Riley, Smith, & Robbins, 1978; Whishaw & Kornelsen, 1993). Interestingly, lesions of the NAc spare the reflexive lordosis response, but attenuate appetitive, proceptive sexual behavior in female rats (Rivas & Mir, 1990, 1991). More targeted techniques such as optogenetic or

pharmacological inhibition of the NAc result in an attenuation of cocaine or natural incentive seeking behaviors (Kelley & Swanson, 1997; Stefanik, Kupchik, Brown, & Kalivas, 2013; Stefanik, Moussawi, et al., 2013; Stuber et al., 2012).

Moreover, appetitive and consummatory behaviors elicit a similar DA surge in the NAc. This has been reported using operant responding protocols for a variety of natural reinforcers (e.g., food and sex; Cacciapaglia et al., 2011; Carelli, Ijames, & Crumling, 2000; Hoebel, 1997; Meisel, Camp, & Robinson, 1993; Mermelstein & Becker, 1995; Nicola, Yun, Wakabayashi, & Fields, 2004; Salamone, Cousins, & Snyder, 1997; Stratford & Kelley, 1997; Wise, 1998) and cocaine (Peoples & West, 1996). Incentive-driven DA release, though, is not uniform throughout the NAc (Baliki et al., 2013; Bassareo & Di Chiara, 1999; Dalton, Phillips, & Floresco, 2014; Di Chiara, 2002; Ikemoto, 2007; Saddoris et al., 2014; Sokolowski et al., 1998). Specifically, both cocaine and natural incentives act preferentially through the shell of the NAc. Rats will readily self-administer cocaine directly into the shell, but not core, of the NAc (Carlezon, Devine, & Wise, 1995; Rodd-Henricks, McKinzie, Li, Murphy, & McBride, 2002), and intact DAergic input in the shell, but not the core, is necessary for the development of conditioned-place preference for cocaine (Sellings, McQuade, & Clarke, 2006). While the activational aspects of naturally rewarding stimuli are not as simple as that of cocaine, studies have determined that the shell of the NAc also mediates their value (Di Chiara, 2002; Numan, Numan, Pliakou, et al., 2005; Sokolowski et al., 1998). For example, experimenters were able to initiate feeding behavior in rats by microinfusing non-NMDA glutamate receptor antagonists or GABA agonists into the shell of the NAc (Kelley & Swanson, 1997; Kelley, 2006) Yet, neural populations in the NAc may respond differently to naturally rewarding stimuli and cocaine. For example, Carelli and Deadwyler (1994) found that there are four subsets of MSNs in the NAc that respond to

cocaine, but one of these subsets does not respond to water reinforcement. Again, there is a great deal of evidence to suggest that cocaine-mediated reward, at least in part, results from the appropriation of neuroanatomical circuits that are responsible for coding the valence of natural rewards.

1.3. HORMONAL MEDIATION OF COCAINE-ASSOCIATED REWARD

Given the role of the mesolimbic DA system in integrating and processing external cues and internal conditions, it is not surprising that the hormonal status of an organism can dictate the response to cocaine. It has been repeatedly demonstrated in women and female animal models that the natural cyclicity of circulating sex steroid hormones (e.g., estrogens and progestogens) that modulate the menstrual or estrus cycles also influences propensity to engage in naturally rewarding behaviors, and cocaine seeking and consumption.

1.3.1. Clinical findings

There are a number of sex differences apparent in the initiation phase of cocaine use, consumption, dependence, and treatment outcomes in humans (e.g., Carroll, Lynch, Roth, Morgan, & Cosgrove, 2004; Hu & Becker, 2008; Lynch et al., 2006), which are discussed above. Differences in circulating sex steroid hormones are, at least in part, responsible for driving these differences (Bobzean, DeNobrega, & Perrotti, 2014; Collins et al., 2007; S M Evans & Foltin, 2006; Suzette M Evans & Foltin, 2010; Lukas et al., 1996; Mendelson et al., 1999; M Sofuoglu et al., 1999). In brief, both men and women have circulating testosterone, estrogens and progestogens that influence almost all physiological systems. Women generally have higher levels of circulating progestogens and estrogens, whereas men have higher levels of circulating testosterone (Mikhail, 1967). Moreover, in women biphasic pulses of estradiol (E_2) and progesterone (P_4)

regulate the menstrual cycle (Mihm, Gangooly, & Muttukrishna, 2011; Silverthorn, 2013; Figure 1.1) and alter their responsivity to salient stimuli (Dreher et al., 2007; Sakaki & Mather, 2012; Terner & de Wit, 2006). The menstrual cycle is typically 28 days in duration and consists of two phases: follicular and luteal. During the mid- to late follicular phase there is a gradual increase in E_2, which peaks about half way through the cycle and then sharply decreases. This peak in E_2 precedes the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary gland, which then stimulate the release of the mature egg from the ovarian follicles. Finally, there is a steady increase in P_4 and a slight rebound of E_2 levels, before they fall to baseline levels at the end of the luteal phase.

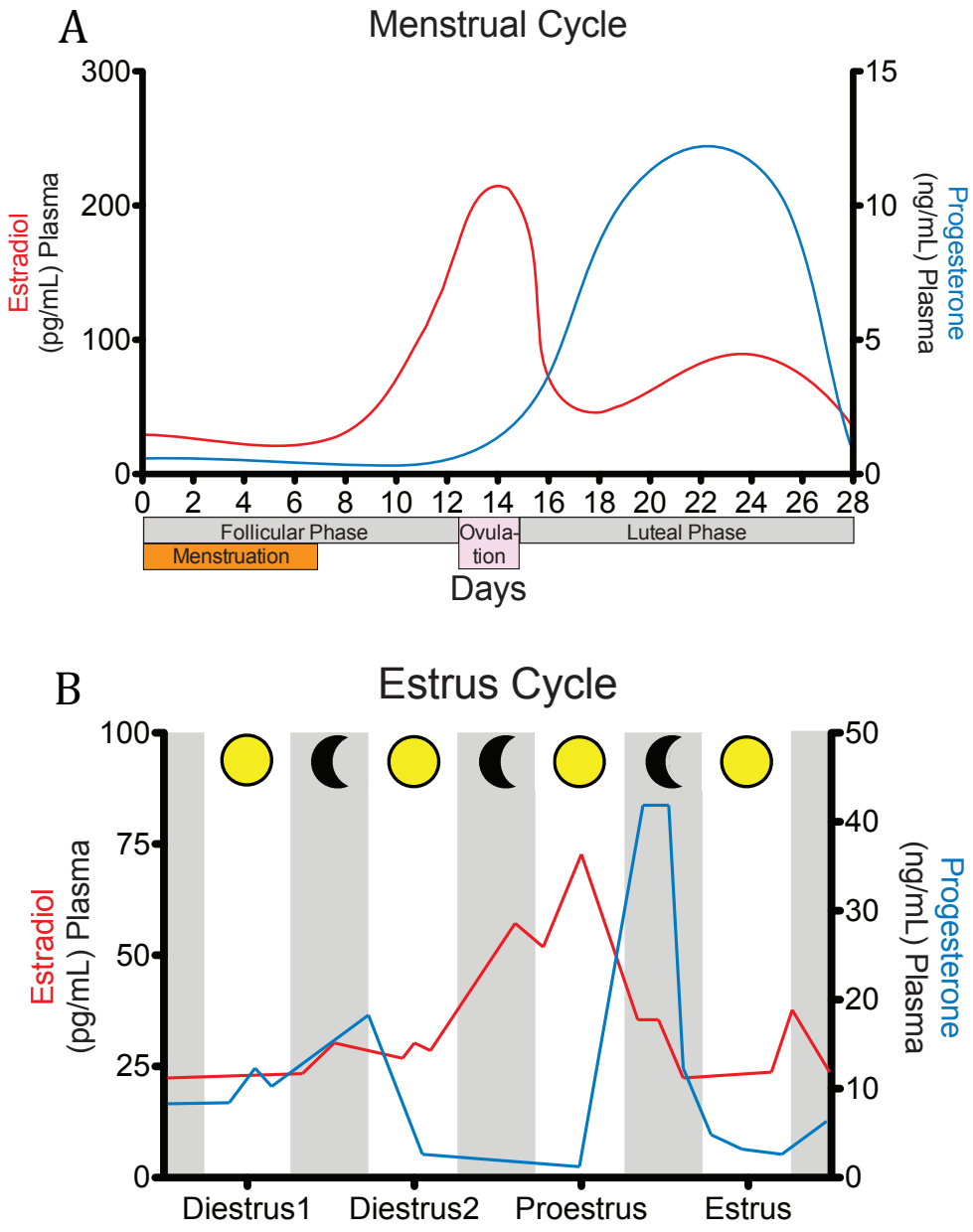

Figure 1.1. Mammalian ovulatory cycles. In both (A) rodents and (B) humans, there is an initial surge in E_2 followed by a surge in P_4 and a secondary, lesser increase in E_2. (A) Adapted from Butcher, Collins and Fugo (1974). (B) Adapted from Stricker et al. (2006).

Circulating levels of E_2 and P_4 influence the subjective effects and

pharmacodynamics of cocaine and other drugs of addiction (see Becker, Perry, & Westenbroek, 2012; Hu & Becker, 2008; Hudson & Stamp, 2011; Quiñones-Jenab, 2006 for extensive reviews). Though the results are not always consistent, many studies have shown that the euphoric or "good drug effect" of cocaine is enhanced during the follicular phase when smoked (Evans & Foltin, 2010; Evans, Haney, & Foltin, 2002; Sofuoglu et al., 1999). Furthermore, during the mid-luteal phase women exhibit attenuated cocaine craving, stress, anxiety, and increase in blood pressure induced by cocaine-associated cues (Sinha et al., 2007). It is unlikely that these phasic differences are attributable to changes in cocaine pharmacokinetics (Collins et al., 2007; Evans & Foltin, 2006; Kosten et al., 1996; Mendelson et al., 1999)

Instead, these differences are likely due to electrophysiological changes in neuronal circuits mediated by fluctuations in circulating levels of E_2 and P_4. Relatively few studies have attempted to examine changes in cocaine-induced reward after administration of exogenous ovarian hormones, and those that have focused mainly on P_4. Overall, studies have found that experiment-administered P_4 during the follicular phase (high E_2) attenuates the "good drug effect" and diminishes the sensitivity of women to cocaine as measured by the ability to "feel the last dose" after smoking (Evans & Foltin, 2006; Sofuoglu, Babb, & Hatsukami, 2001; Sofuoglu, Babb, & Hatsukami, 2002) and i.v. administration (Sofuoglu, Mitchell, & Kosten, 2004). Salivary P_4 is negatively correlated with the euphoric effects of a similar psychostimulant, D-amphetamine, whereas salivary E_2 is positively correlated with subjective reports of euphoria (White, Justice, & de Wit, 2002). The preceding studies only examined the subjective, self-reported effects of ovarian hormones on cocaine use. The only study that has assessed the influence of ovarian hormones on motivation to consume cocaine found that exogenous P_4 does not attenuate the propensity to use cocaine at any phase of the menstrual cycle

(Reed, Evans, Bedi, Rubin, & Foltin, 2011).

Of the various challenges involved in completing such studies using human participants, one particular confound arises. Namely, previous research has revealed that cocaine can disrupt the cyclicity of hormone pulses. Cocaine consumption stimulates LH, which can disrupt the menstrual cycle (Mello et al., 2007; Mendelson et al., 1999; Sofuoglu et al., 1999) and can lead to amenorrhea and infertility (Cocores, Dackis, & Gold, 1986). Furthermore, abstinence from chronic cocaine consumption has been shown to increase circulating P_4 and cortisol levels for at least one month (Fox, Hong, Paliwal, Morgan, & Sinha, 2008). These findings are interesting in their own right and suggest that cocaine may directly influence the circulating sex steroid hormones that, in turn, mediate responsivity to cocaine.

1.3.2. Preclinical findings

To compensate for the scarcity of data from humans, preclinical models exploring the effects hormonal cyclicity on cocaine-associated behaviors have been able to fill in the gaps. The menstrual cycle of the monkey closely mirrors that of women (Goodman, Descalzi, Johnson, & Hodgen, 1977), while rodents have an estrus cycle of 4 to 5 days (Butcher, Collins, Fugo, & Virginia, 2013; see figure 1.1.B). The longest phase of the estrus cycle is the diestrus phase, which lasts about 2 days. During this stage both of the ovarian hormones are at their lowest levels, with a progressive increase in P_4 on diestrus day 1 and a gradual decrease in P_4 during diestrus day 2. Similar to the menstrual cycle, there is a sharp rise in E_2 before ovulation – the proestrus phase. This increase is followed closely by an increase in LH, FSH, and P_4 that begins late in the proestrus phase and diminishes rapidly over the estrus phase. It is during the estrus phase of the cycle that female rodents display signs of sexual receptivity (Beach, Etkin, & Rasquin, 1942;

Boling & Blandau, 1939; Powers & Valenstein, 1972). In both monkeys and rodents the cyclic nature of ovulation and coinciding levels of ovarian hormones influence the different phases of cocaine use.

There are differences in behavioral responses to cocaine between stages of the ovulatory cycle at all phases of drug addiction. A larger percentage of female rats acquire cocaine self-administration during estrus compared to non-estrus phases (Lynch & Carroll, 2000). On the contrary, female monkeys do not show phasic differences in cocaine acquisition or consumption (Cooper, Foltin, & Evans, 2012; Suzette M Evans & Foltin, 2010).

After the acquisition phase, the subjects generally establish a stable pattern of cocaine consumption defined as the maintenance phase of cocaine abuse. During this phase female rats lever-press for cocaine on a progressive ratio schedule had significantly higher breakpoints and more rapid escalation of cocaine intake during the estrus phase (Lynch & Carroll, 2000; Roberts et al., 1989). Examining cocaine seeking (lever pressing) during the last day of a fixed ratio self-administration schedule, Feltenstein and See (2007) found that rats exhibited the highest rates of cocaine-seeking behaviors during estrus (low P_4), and the lowest rates of cocaine seeking behavior during late proestrus (high P_4). In a similar vein, female monkeys have higher progressive ratio breakpoints during the follicular phase (high E_2; Mello et al., 2007).

The hormonal influences on extinction and drug-primed reinstatement of cocaine seeking are similar to those seen during the acquisition, escalation, and maintenance phases. Namely, operant responding is elevated during the extinction phase (Feltenstein & See, 2007; Kerstetter et al., 2008) and drug-primed reacquisition phase (Feltenstein & See, 2007; Kerstetter et al., 2008; Kippin et al., 2005) in the estrus stage of the cycle.

As is the case for humans, cycle-mediated differences in cocaine

pharmacokinetics are an unlikely explanation for the antecedent findings. Studies have found that there are no changes in cocaine pharmacokinetics as a function of menstrual cycle in non-human primates (Evans & Foltin, 2004, 2006; Mello, Mendelson, Kelly, & Bowen, 2000), or as a function of the estrus cycle phase in rats (Festa et al., 2004).

The phasic and complex nature of the estrus and menstrual cycles make it difficult to determine exactly which hormones influence instrumental learning or operant responding at the different stages of cocaine dependence. For these reasons, many studies opt to use hormone replacement protocols in ovariectomized females. Such hormone replacement studies suggest that, generally, E_2 enhances and P_4 attenuates cocaine-mediated conditioned approach behavior (Hu, Crombag, Robinson, & Becker, 2004; Jackson et al., 2006; Kerstetter et al., 2012; Martinez, Peterson, Meisel, & Mermelstein, 2014; Peris, Decambre, Coleman-Hardee, & Simpkins, 1991; Perry, Westenbroek, & Becker, 2013; Ramôa, Doyle, Naim, & Lynch, 2013; van Swearingen et al., 2013; Walker et al., 2001; but see Segarra et al., 2010). Once again, the majority of the phases of cocaine dependence are influenced by hormone replacement.

The literature indicates that E_2 enhances acquisition of cocaine self-administration, but the combined effect of E_2 and P_4 is less clear. For example, several studies have found that E_2 enhances rates at which female rats acquire operant responding to cocaine (Hu et al., 2004; Lynch et al., 2001; Perry et al., 2013), and another study demonstrated that E_2 alone leads to faster acquisition than combined E_2 and P_4 replacement (Jackson et al., 2006). While classical conditioning paradigms examining cocaine acquisition also find that E_2 leads to increased responding compared to vehicle (Frye & Rhodes, 2006; Segarra et al., 2010, 2014; but see Twining, Tuscher, Doncheck, Frick, & Mueller, 2013), they also suggest that E_2 and P_4 together lead to higher cocaine seeking than E_2 alone (Russo et al., 2003). This discrepancy may be attributable to

differences in drug administration route (i.v. vs. i.p.), timing of hormone injections (Bobzean, Dennis, & Perrotti, 2014), or the conditioning paradigms themselves (Tzschentke, 2007). During the maintenance phase, systemic P_4 treatment leads to a more gradual escalation of cocaine consumption compared to vehicle treatment (Larson, Anker, Gliddon, Fons, & Carroll, 2007), and systemic E_2 increases the number of times rats choose cocaine over food in an operant responding task (Kerstetter et al., 2012). These behaviors may be dependent on the length of access to cocaine during the acquisition phase. Ramoa and colleagues (2013) showed that E_2 enhances cocaine seeking during maintenance, but only when female rats are given longer access to cocaine during acquisition. E_2 also enhances behavioral sensitization to cocaine (Martinez et al., 2014; Peris et al., 1991), and leads to a more rapid extinction of cocaine seeking behaviors (Twining et al., 2013). Finally, P_4 and its metabolites attenuate drug-primed reinstatement of cocaine seeking (Anker & Carroll, 2010; Feltenstein, Byrd, Henderson, & See, 2009).

In all, the research suggests that animals with elevated E_2 levels, whether due to natural cyclicity or hormone replacement, acquire cocaine responding faster and at higher rates, which may or may not be further enhanced by concurrent treatment with P_4. Moreover, elevated levels of E_2 and enhance responding during the maintenance phase and exacerbate escalation of cocaine consumption, but also accelerate extinction of cocaine seeking behaviors. At the same time, P_4 may attenuate acquisition and drug-primed reinstatement. Discrepancies in the literature are likely due to differences in methodologies. As in humans, evidence suggesting that chronic cocaine use can disrupt the reproductive cycle in rodents (Chen & Vandenbergh, 1994), and non-human primates (Mello et al., 1993; Potter et al., 1999) further complicates the study of hormonal effects on cocaine-taking behavior. Regardless, research has still been able to identify pertinent

roles of ovarian hormones in modulating cocaine use and abuse.

1.3.3. Hormones and the mesolimbic DA system

One possible way in which ovarian hormones might influence the salience of cocaine in females is through direct actions on the mesolimbic DA system. This theory stems from observations in animals and humans that systemic sex steroid hormones affect psychostimulant-mediated DA release in the striatum (Becker, 1999; Di Paolo, 1994). E_2 enhances cocaine-mediated tonic DA levels in the ventral striatum (Cummings, Jagannathan, Jackson, & Becker, 2014). Moreover, systemic E_2 decreases striatal DAT density, enhances DA synthesis and degredation (McArthur, McHale, & Gillies, 2007; Pasqualini, Olivier, Guibert, Frain, & Leviel, 2002), and downregulates DA binding to D_2Rs in the striatum (Bazzett & Becker, 1994). *In vitro*, direct pulsatile application of E_2 to striatal slices induces DA release (Becker, 1990) and enhances K^+-mediated DA release (Thompson & Moss, 1994). Patch clamp analysis of ion transfer across the membrane in disassociated MSNs demonstrated that there is a prompt diminution of Ca^{++} currents in response to acute E_2 (Mermelstein & Becker, 1995). Interestingly, E_2 administered inside the cytosol does not reduce the Ca^{++} current, whereas E_2 bound to bovine serum albumin, which does not allow it to cross the cell membrane, most effectively decreases the current. These results suggest that E_2 is acting through a membrane-associated protein that is able to rapidly influence ion permeability in the striatum, and the resultant decrease in Ca^{++} current attenuates GABA release. Accordingly, studies have confirmed a high density of G protein-coupled estrogen receptor 1 (GPER; formally known as GPR30) in the striatum (Brailoiu et al., 2007). Since prior experiments show that the striatum has a paucity of ERα and ERβ (DonCarlos, Monroy, & Morrell, 1991; Mitra et al., 2003; Österlund, 1998), the presence

of GPER provides a mechanism through which E_2 can act in the striatum.

Similarly, PRs in the mesolimbic DA system are sparse (Blaustein, Tetel, Nielsen Ricciardi, Delville, & Turcotte, 1994; MacLusky & McEwen, 1978; Warembourg & Leroy, 2004); but P_4 has been shown to influence electrophysiological and neurochemical activity (Hudson & Stamp, 2011). Studies in women have demonstrated that exogenous P_4 attenuates the euphoric effects of smoked cocaine (Evans & Foltin, 2006; Sofuoglu et al., 2004), suggesting that P_4 somehow decreases response to or release of DA in the ventral striatum. At the same time, the direct action of P_4 on activity in the mesolimbic DA system is not as well defined as it is for E_2, and reports have claimed opposing actions. In the VTA, microinfusions of P_4 increase activity of neurons within 60 seconds (Rose, 1990). Systemic P_4 in ovariectomized females increases the basal firing rate of VTA DA neurons and simultaneously attenuates cocaine-mediated firing rate and bursting activity compared to vehicle treatment (Zhang, Yang, Yang, Jin, & Zhen, 2008). These phenomena may be a function of a P_4-mediated increase in basal DA and 5-HT release in the VTA (Russo et al., 2003). Interestingly, this study did not show an effect of P_4 on DA release in cocaine treated animals. Instead, observed effects of P_4 in the VTA may stem from its influence on the striatum. Actions of P_4 in the VTA may also be a function of its neuroactive metabolites (Frye, 2007). The metabolite allopregnanolone (3α,5α-THP) is a positive allosteric modulator of $GABA_A$ receptors ($GABA_AR$; Belelli, Casula, Ling, & Lambert, 2002) and a negative allosteric modulator of the 5-HT_3 receptor (Wetzel et al., 1998), and has been shown to influence activity in the VTA (Frye, 2007). It is also possible, then, that an increase in GABA sensitivity, via modulation of $GABA_AR$ by allopregnanolone may reduce activity in GABA-sensitive DA neurons in the VTA, thus decreasing DA release in the striatum.

Experimental data does not support such a simple role for neuroactive metabolites

or P_4 in general. Researchers have reported that E_2-primed animals given systemic P_4 exhibit an enhanced DA response in the NAc after psychostimulant administration (Becker & Rudick, 1999; Dluzen & Ramirez, 1984, 1987, 1989, 1990, 1991; Russo, Jenab, et al., 2003). Additionally, *in vivo* treatment with P_4 does not influence D_2R binding in the striatum, unlike systemic E_2; suggesting that, even though E_2+P_4 treatment may increase drug-induced DA release, it does not influence striatal sensitivity to DA (Lévesque & Di Paolo, 1991). To complicate this issue further, other studies have shown that P_4 treatment alone decreases the number of neurons in the NAc containing D_2-like DA receptors (Fernández-Ruiz, Amor, & Ramos, 1989), and actually decreases DA release in striatal slices (Peris et al., 1991). Though this discrepancy is difficult to reconcile, there is another more promising avenue through which P_4 may mediate the response of the striatum to DA. Just as E_2 may influence NAc DA signaling through membrane hormone receptors, P_4 may also act primarily through membrane-associated proteins (Ke & Ramirez, 1990; Ramirez, Zheng, & Siddique, 1996). Several recently discovered membrane-associated progesterone receptors are indeed present in the VTA and the striatum (Intlekofer & Petersen, 2011b; Pang, Dong, & Thomas, 2013; Petersen et al., 2013). Signals mediated by these membrane progesterone receptors have been implicated in regulating female sexual behaviors (Mani & Portillo, 2010), but the brain regions through which they act are not known.

Despite the lack of traditional nuclear receptors for E_2 and P_4 in the VTA and NAc, these hormones work, often in tandem with one another, to modulate the activity and neurochemistry of the mesolimbic DA system. Both ovarian hormones increase DA release in the medial Ventral striatum, but the P_4-mediated increase seems to conflict with clinical and preclinical studies showing that P_4 generally attenuates the reinforcing and rewarding aspects of cocaine. In turn, the actions of these ovarian hormones on the NAc

and VTA may be mediated by novel membrane-associated receptors, associated neuroactive metabolites, or regulation of secondary or tertiary neural systems downstream.

1.4. A ROLE FOR THE MPOA IN COCAINE-MEDIATED REWARD

While circulating sex steroid hormones directly influence the functioning of the mesolimbic reward system, it is also likely that they modulate its activity indirectly through connections with hypothalamic regions essential for regulating and integrating the hormonal milieu. A particularly attractive hypothalamic candidate for such modulation is the medial preoptic area (mPOA). The mPOA was the first brain region discovered to contain a sexual dimorphic nucleus (SDN; Gorski, Gordon, Shryne, Southam, & Angeles, 1978; Gorski, Harlan, Jacobson, Shryne, & Southam, 1980). The SDN is an ovoid cluster of sizable perikarya that is located near the centromedial portion of the mPOA and is about twice as large in males as it is in females. The mPOA is one of the most rostral hypothalamic structures, is adjacent to the third cerebral ventricle, and lies between the anterior commisure and the optic chiasm.

As with most neural nodes, the mPOA has reciprocal, afferent, and efferent connections with a multitude of neuroanatomically distinct brain regions. Tract-tracing studies done by Simerly and Swanson (1986, 1988) demonstrate that the mPOA has a dense network of reciprocal connections with the lateral septum (LS), BNST, medial amygdala (MeA), arcuate nucleus (ARC), and periventricular nucleus of the hypothalamus (PVN). Regions that send the majority of the unilateral signals to the mPOA are the ventromedial hypothalamus (VMH), the amygdalo-hippocampal area, and the lateral PBP of the VTA. Finally, key regions that receive information from the mPOA include the lateral preoptic area, PN of the VTA, the medial PBP of the VTA, and the

periaqueductal gray (PAG). Qualitative studies of preoptic connections, particularly with subregions of the VTA, suggest that the mPOA may influence motivation and reward.

Experiments employing lesions and electrical stimulation of the mPOA to examine its influence on naturally rewarding behaviors have provided the strongest data in support of a role for the mPOA in mediating goal-oriented behaviors. In both sexes, the mPOA has been shown to regulate a variety of behaviors and autonomic functions, which include, but are not limited to, thermoregulation (Bicego, Barros, & Branco, 2007; Boulant, 1981; Kumar, Vetrivelan, & Mallick, 2007), hypovolemic thirst (Bourque, Oliet, & Richard, 1994) and feeding (Leibowitz et al., 2007; Patterson et al., 2006). In males, mPOA lesion studies have mostly focused on the resultant attenuation of sexual behaviors (Alekseyenko, Waters, Zhou, & Baum, 2007; Dominguez, 2009; Dominguez & Hull, 2005; Liu, Salamone, & Sachs, 1997; Paredes, Tzschentke, & Nakach, 1998; Powers, Newman, & Bergondy, 1987; Will, Hull, & Dominguez, 2014). Meanwhile, lesions of the female mPOA influence both sexual behavior (Guarraci & Clark, 2006; Paredes et al., 1998; Whitney, 1986; Yahr & Greene, 1992) and maternal behaviors (Lee et al., 1999; Numan, Numan, Schwarz, et al., 2005; Tsuneoka et al., 2013). Interestingly, ablations of the mPOA have a dichotomous effect on appetitive and consummatory sexual behaviors in female rats. In particular, lesions of the mPOA facilitate lordosis (Rodriguez-Sierra & Terasawa, 1979; Whitney, 1986), whereas they attenuate proceptive behaviors and paced mating (Whitney, 1986; Yang & Clements, 2000) and disrupt partner preference (Guarraci & Clark, 2006). The cause of this disparity between control of appetitive and consummatory behaviors is not fully understood. One possibility is that it may be a function of variations in lesion placement. Balthazart and Ball (2007) proposed that the rostral mPOA is necessary for the expression of both appetitive and consummatory sexual behavior, whereas the caudal mPOA regulates only consummatory

sexual behaviors.

Maternal behaviors, on the other hand, are uniformly attenuated by mPOA lesions (Numan & Smith, 1984; Tsuneoka et al., 2013) and facilitated by electrical stimulation (Morgan, Watchus, Milgram, & Fleming, 1999). For example, ablation of the mPOA diminishes both operant responding for pups (Lee et al., 1999), and classical conditioning to pup-associated environments (Pereira & Morrell, 2010, 2011). An early lesion study also demonstrated that the projection from the mPOA to the VTA was necessary for the expression of maternal behavior (Numan & Smith, 1984). Thus, in females, appropriate behavioral responses to a variety of naturally reinforcing stimuli are dependent on the mPOA and possibly its interactions with the VTA.

While ablation and stimulation studies gave insight into the function of the mPOA, other studies have expanded on the possible neurochemical mechanism and neuroanatomical connections involved. GABA, an inhibitory amino acid, is the principle neurotransmitter produced by perikarya in the mPOA (Herbison, Augood, & McGowan, 1992; Herbison, Augood, Simonian, & Chapman, 1995; Simmons & Yahr, 2003; Tsuneoka et al., 2013). Indeed, there are about three times as many GABA-producing cells as there are glutamate-producing (excitatory amino acid) cells in the mPOA (Tsuneoka et al., 2013). Furthermore, the vast majority of cells in the mPOA that project to the VTA are GABAergic (Tobiansky et al., 2013), and many of these are activated during sexual behavior or access to pups (Simmons, Hoffman, & Yahr, 2011; Tsuneoka et al., 2013). On the other hand, there are minimal glutamate-containing efferents (Geisler, Derst, Veh, & Zahm, 2007). These reports suggest that the mPOA may play a role in inhibiting activity within the VTA. Studies examining neuropeptides produced within the mPOA further suggest that the mPOA inhibits downstream brain regions. A comprehensive study done by Tsuneoka and colleagues (2013) also showed that galanin

neurons, along with the neuropeptides neurotensin and neurokinin B, were activated by exposure to pups in females as well. Though each of these neuropeptides has a different mechanism of action, when microinjected into the VTA, they all enhance DA release in the NAc (Ericson & Ahlenius, 1999; Kalivas, Widerlov, Stanley, Breese, & Prange, 1983; Marco, Thirion, Mons, & Bougault, 1998).

Considering that the mPOA also receives DAergic input from the VTA along with afferents from adjacent DAergic regions (Miller & Lonstein, 2009), it is not surprising that DA signaling in the mPOA influences sex and maternal behaviors. DA increases in the mPOA during bouts of copulation in female rats (Matuszewich, Lorrain, & Hull, 2000) and when given access to their pups after a short period of separation (Hansen, Bergvall, & Nyiredi, 1993). In a pair of studies, Dean-Graham and Pfaus (2010, 2012) found that DA receptor agonists and antagonists microinjected into the mPOA differentially influence receptive and proceptive female sexual behaviors. They posited that the D_1R-to-D_2R ratio in the mPOA dictates the behavioral output, and that sex steroid hormones may shift this ratio in favor of one receptor over the other.

Although sex steroid hormones may act in part by shifting the balance of DA receptor activation, receptors for the hormones themselves are also found at high densities throughout the mPOA. As discussed above, the literature has focused on two main ovarian hormones in the modulation of reinforcing behaviors: E_2 and P_4. The mPOA has a higher concentration of ERα and PR than mesocorticolimbic brain regions (DonCarlos et al., 1991; Intlekofer & Petersen, 2011; Merchenthaler, Lane, Numan, & Dellovade, 2004; Österlund, 1998; Parsons, Rainbow, MacLusky, & McEwen, 1982; Quadros, 2002; Simerly, Chang, Muramatsu, & Swanson, 1990; Warembourg & Leroy, 2004). Concentrations of these SSHRs in the mPOA vary throughout the ovulatory cycles (Shughrue, Bushnell, & Dorsa, 1992), during pregnancy (Koch and Ehret, 1989; Mann

and Babb, 2005), and following parturition (Ehret & Buckenmaier, 1995; Francis, Meddle, Bishop, & Russell, 2002; Tsuneoka et al., 2013). These changes are reflected in alterations of electrophysiological properties (Kelly, Moss, Dudley, & Fawcett, 1977) and behavioral output (Champagne, Weaver, Diorio, Sharma, & Meaney, 2003). Implantation of E_2 crystals or microinfusions of E_2 into the mPOA enhances certain maternal behaviors (Fahrbach & Pfaff, 1986; Numan, Rosenblatt, & Komisaruk, 1977), but reduces food intake (Santollo, Torregrossa, & Eckel, 2011). At the same time, virus-mediated downregulation of ERα in the mPOA severely attenuates maternal behavior and consummatory sexual behaviors (Ribeiro et al., 2012; Spiteri, Ogawa, Musatov, Pfaff, & Ågmo, 2012), but increases sex incentive approach behaviors (Spiteri et al., 2012). The regulatory actions of E_2 in the mPOA on these behaviors may be through the result of ERα activation in perikarya that project to the VTA (Fahrbach et al., 1986). The only study, to my knowledge, examining microinfusions of P_4 into the mPOA found that they facilitated lordosis in E_2-primed rats (Beyer & Gonzalez-Mariscal, 1989). Unfortunately, no studies have examined how PRs in the mPOA directly influence the expression of naturally rewarding behaviors in females. Together, the aforementioned reports suggest that E_2, through its actions on ERα, enhance consummatory behaviors, but attenuate appetitive behaviors, while the actions of P_4 in the mPOA on these behaviors require further examination.

Viewed collectively, neuroanatomical, neurochemical, and neurohormonal evidence supports the role of the mPOA as a modulatory node for influencing naturally rewarding behaviors. Considering the its DAergic input and its role in regulating maternal and sex behaviors, and preoptic connectivity with the mesolimbic system, it stands to reason that the mPOA may also influence cocaine-associated behaviors. Indeed, there have been several studies examining how the mPOA mediates cocaine-associated

behaviors (Mattson, Williams, Rosenblatt, & Morrell, 2001; Mattson, Williams, Rosenblatt, & Morrell, 2003; Pereira & Morrell, 2010, 2011; Seip & Morrell, 2007; Seip et al., 2008), and how cocaine mediates activity in and natural behaviors through the mPOA (Mattson & Morrell, 2005; Pfaus et al., 2010; Vernotica, Rosenblatt, & Morrell, 1999; Zahm et al., 2010). Systemic cocaine was known to impair both female sexual (Pfaus et al., 2010) and maternal behaviors (Nephew & Febo, 2012), but Vernotica and colleagues (1999) were the first to suggest that cocaine may act specifically through the mPOA to inhibit maternal behavior. More recent studies show that cocaine-associated cues increase putative cellular activity in the mPOA (Mattson & Morrell, 2005). In a subsequent study from the same laboratory found that transient inactivation of the mPOA during conditioning cycles to pups or cocaine attenuated maternal behavior during the early postpartum period, but paradoxically enhanced maternal behavior during the later postpartum period (Pereira & Morrell, 2011). Moreover, the group found that transient inhibition of the mPOA in postpartum dams during CPP acquisition exhibited a preference for the cocaine-associated chamber over the pup-associated chamber in a conditioned place preference paradigm (Pereira & Morrell, 2010, 2011).

These results imply that cocaine may influence activity of the mPOA and that the mPOA may regulate cocaine-seeking behaviors. The actions of cocaine in the mPOA are likely mediated by DA receptors. Furthermore, considering the abundance of inhibitory GABA neurons in the mPOA, its connections to the VTA, and its role in mediating cocaine-seeking behavior, cocaine's enhancement of activity in the mPOA likely increases activity of DAergic VTA neurons (see Illustration 1). Finally, circulating sex steroid hormones may influence the activational patterns of preoptotegmental efferents in response to cocaine, but further studies must be done examining this direct relationship.

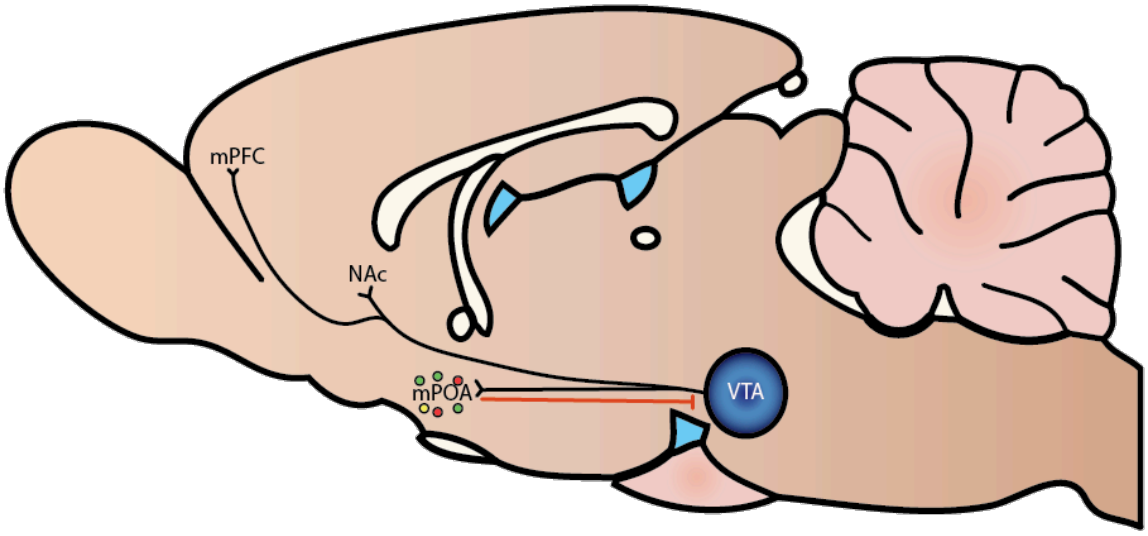

Illustration 1: The proposed preoptotegmentostriatal circuitry. This circuit involves the mPOA in mediating the rewarding and reinforcing aspects of natural incentives and cocaine. Multicolored circles within the mPOA symbolize a dense concentration of different sex steroid hormones

1.5. SUMMARY AND CONCLUSIONS

Overall, preclinical and clinical research has shown clear sex differences in cocaine-associated behaviors and provides strong evidence that there is a neuroendocrine regulation of cocaine-mediated reinforcement. Females display higher rates of acquisition and maintenance, increased resilience to extinction and higher responding during reinstatement. Hormonal cyclicity and replacement in females also influences cocaine-associated reinforcement. While the mesolimbic system and associated structures are somewhat sensitive to circulating hormones, there are hypothalamic regions that are heavily regulated by SSHs, interact with the mesolimbic system, and are pertinent for processing natural rewards. In particular, the mPOA has some of the highest

concentrations of SSHRs in the brain, interacts directly with the VTA, and is directly involved in the expression of naturally rewarding behaviors. Considering that cocaine appropriates neural circuits responsible for processing natural reward, it is likely that the mPOA is involved in mediating cocaine-associated neurochemistry and behaviors.

Chapter 2: Establishing mPOA connectivity to the mesolimbic reward system: neuroanatomy, phenotyping, and functionality[1]

2.1. ABSTRACT

The medial preoptic area (mPOA) is involved in the regulation of naturally rewarding behaviors, including maternal and sexual behaviors. The mPOA also influences drug-mediated behaviors, such as cocaine acquisition. Tract-tracing studies have shown that the mPOA may influence the mesolimbic reward system via projections to the ventral tegmental area (VTA). Yet, the mechanisms through which the mPOA influences the incentive salience of natural and non-natural rewards remain relatively unexplored. Given its putative inhibitory interactions with the mesolimbic reward system and its importance for naturally and non-naturally rewarding behaviors, we sought to determine whether the mPOA influences the reward system via GABAergic projections on dopamine (DA)-producing cells in the VTA, and whether these efferents were DA- or hormone-sensitive. Moreover, we sought to determine whether cocaine had activational effects on these efferents. Approximately 68% of mPOA-VTA efferents contain γ-aminobutyric acid (GABA), over 75% are sensitive to DA as evidenced by co-localization with DA receptors, and nearly 60% of these contain both DA receptors and GABA. Subsequently, we show that a high proportion of preoptotegmental perikarya contained SSHRs. In particular, progesterone receptor membrane component 1 (PGRMC1) was found in significantly more preoptic neurons (>60%) than the nuclear progestogen receptor (<1%). Nuclear estrogen receptors (ERα) and membrane estrogen receptors (GPER) were present at similar levels in preoptotegmental perikarya in the

[1]Portions of text in this section are excerpted from Tobiansky DJ, Roma PG, Hattori T, Will RG, Nutsch VL, Dominguez JM, Behavioral Neuroscience 127(2), 293-302. PGR assisted in establishing the conditioned place preference paradigm in our lab. TH, RGW, VLN and JMD were involved in edits and assisted in surgery.

central mPOA (~60%). Finally, a large proportion of these mPOA-to-VTA efferents appose DArgic cells in the VTA that then project to the shell of the nucleus accumbens (~45%). These findings further elucidate the role of brain regions not typically recognized as part of the reward circuitry in modulating the behavioral response to drugs of addiction. They also suggest a mechanism by which SSHs may act through indirect means to modulate the salience of these drugs.

2.2. INTRODUCTION

Rates of cocaine abuse have increased among women and adolescent females in the last few decades (Zilberman, Tavares, & El-Guebaly, 2003). Women also have higher cue reactivity and much higher levels of cocaine craving during periods of abstinence (see Lynch et al., 2002 for review). Furthermore, female rats acquire cocaine self-administration faster and at lower doses than males (Lynch & Carroll, 1999; Lynch, 2008; Russo, Jenab, et al., 2003), and have an increased locomotor response (Sell, Scalzitti, Thomas, & Cunningham, 2000) relative to males. Yet, many rehabilitation paradigms and treatments rely on treating addiction in the same manner regardless of sex, and worse yet treatment designs have been based on primarily male-centric data (Gillies & Mcarthur, 2010; Lynch et al., 2002). Meanwhile, there is overwhelming evidence across species that ovarian hormones modulate the rewarding properties and pharmacokinetics of addictive psychostimulants (Bowman et al., 1999; Lukas et al., 1996; Niyomchai et al., 2006; see Quiñones-Jenab, 2006 for review; Zhang et al., 2008). For example, the subjective value of psychostimulants differs across the menstrual cycle in women (Collins et al., 2007; Evans et al., 2002; Jeffcoat, Perez-Reyes, Hill, Sadler, & Cook, 1989), and treatment with exogenous progesterone (P_4) attenuates the hedonic value of smoked cocaine (Evans & Foltin, 2006; Sofuoglu et al., 2004). Female rats also

experience similar hormone-mediated differences in conditioned approach behavior depending on the stage of their estrus cycle (Caine et al., 2004; Feltenstein et al., 2009; Feltenstein & See, 2007). This suggests that there is a need for a further understanding of how sex steroid hormones (SSHs) and their corresponding receptors (SSHRs) influence the rewarding and addictive properties of psychomotor simulants.

The neurocircuitry underlying psychostimulant-associated behavior is well established. Phasic dopamine (DA) from neurons originating in the ventral tegmental area (VTA) is released in a number of subcortical and cortical regions (Chen, Tsai, Yeh, Tai, & Tsai, 2008; Wise, 1996). Projections from the VTA to medium spiny neurons in the ventral striatum, otherwise known as the mesolimbic DA system, are thought to be the principal mediators of drug-associated behaviors (see Ikemoto & Bonci, 2014 for review). Yet, there are a number of other areas (e.g., periaqueductal gray, basal lateral amygdala, lateral habenula, locus coeruleus) that modulate the output of these DArgic neurons (see Ikemoto & Bonci, 2014; Jhou et al., 2013 for review). While SSHs have been shown to act directly on the VTA and ventral striatum (Frye, Walf, Kohtz, & Zhu, 2013; Frye, 2007; Schultz et al., 2009) and modulate their cellular activity (Zhang et al., 2008), other brain regions (e.g., ventral medial hypothalamus and preoptic areas) are more sensitive to neurosteroids and interact with the VTA (Adamantidis et al., 2011; Chiba & Murata, 1985; Fahrbach et al., 1986; Jhou et al., 2013; Simerly & Swanson, 1988; Stopper & Floresco, 2014; Swanson, Mogenson, & Simerly, 1987; Tobiansky et al., 2013). These diencephalic nodes are essential for the integration and regulation of the hormonal milieu (Blaustein & Erskine, 2002; Spratt & Herbison, 1997) and have much higher concentrations of SSHRs (Hazell et al., 2009; Intlekofer & Petersen, 2011; Liu & Arbogast, 2009; Mitra et al., 2003; Simerly et al., 1990).

Hypothalamic areas that interact with the mesolimbic system are also essential for the expression of naturally rewarding behaviors (Dominguez & Hull, 2005; Hull, Du, Lorrain, & Matuszewich, 1995; Lonstein, Dominguez, Putnam, De Vries, & Hull, 2003). One area in particular, the medial preoptic area (mPOA), regulates maternal behavior (Numan & Smith, 1984; Numan, Numan, Pliakou, et al., 2005; Stack, Balakrishnan, Numan, & Numan, 2002; Stolzenberg & Numan, 2010; Tsuneoka et al., 2013) and reproductive behaviors in females (Graham & Pfaus, 2010, 2012). Chemical inhibition of the mPOA attenuates conditioned behaviors associated with natural reinforcers (Pereira & Morrell, 2010). Considering that drugs of addiction appropriate neurocircuitry involved in regulating naturally rewarding behaviors (see Hedges, Staffend, & Meisel, 2010 for review), and the mPOA is necessary for the expression of naturally reinforcing behaviors, it is not surprising that the mPOA also modulates psychostimulant responses. Projections from the mPOA to the VTA contain high concentrations of DA receptors, suggesting that they are responsive to alterations in extracellular DA levels, and thus, the actions of cocaine (Tobiansky et al., 2013). Likewise, removal of the mPOA facilitates place preference and increases activity of mesolimbic regions in rats when cocaine is administered systemically (Tobiansky et al. 2013). With one of the highest levels of SSHRs in the brain, direct interaction with the VTA, and influence on conditioned approach behavior, the mPOA is a logical candidate for a locus of hormonal modulation of the rewarding properties of dopamimetic drugs.

The purpose of this experiment was to further examine anatomical and functional connections from the mPOA to the VTA and determine the role that SSHs might have in regulating these cells. We assessed (1) the subregional distribution of perikarya in the mPOA projecting to the VTA, (2) the subregional distribution of mPOA outputs in the VTA, (3) the presence of the inhibitory transmitter, GABA, and DA receptors in

preoptotegmental perikarya, (4) the sex steroid hormone receptor profile of these cells, (5) the influence of cocaine on the activity of these cells using molecular markers of activation, and (6) the extent of interactions that mPOA-to-VTA fibers have with DArgic cells in the VTA that then project to the nucleus accumbens (NAc).

2.3. Materials and Methods

2.3.1. Subjects

Adult female Sprague-Dawley rats (PN 65-74, 200-224g; N = 42; Harlan Laboratories, Indianapolis, IN) were double housed in a temperature-controlled room (22°C, 40 to 50% humidity) and subjected to a reverse light/dark cycle (14 hours light/10 hours dark; lights off at 10 a.m.). Each female was ovariectomized at the time of the intracranial tract-tracer injection and received an intrascapular estradiol silastic capsule implant (5% 17-β-estradiol benzoate, 95% cholesterol; 12 mm in length; 1.98 mm I.D. X 3.18mm O.D.; Dow Corning, Midland, MI). Animal husbandry, surgeries, and experiments were approved by the Institutional Animal Care and Use Committee at The University of Texas at Austin and were in accordance with the National Health Guidelines for the Use of Animals in Research.

2.3.2. Surgery

2.3.2.1. Experiment 1

An intraperitoneal (i.p.) injection of a ketamine hydrochloride (50mg/kg; Animal Health Intl. Greeley, CO) and xylazine hydrochloride (4mg/kg; Animal Health Intl. Greeley, CO) cocktail was used to anesthetize the rats (n = 30). They were then placed into a stereotaxic apparatus, the skull was exposed via a mid-line incision, and a portion of the skull was removed above the region of interest. A pulled glass electrode (opening, ~20μm in diameter) containing the retrograde tract tracer Fluorogold (FLG;

Fluorochrome, LLC, Denver, CO) was lowered into the VTA (coordinates determined from Paxinos & Watson, 2007; AP, -6.2 mm; ML, +0.7 mm; DV, -8.2 mm). A silver wire inserted into the glass electrode, such that it contacted the FLG solution, was connected to an iontophoretic current source (Midgard Precision Current Source, Stoelting Co., Wood Dale, IL). Current-induced FLG injections occurred immediately after the electrode was in position (+7μA, 7 seconds-on, 1 second-off pulses for 5min; Tobiansky et al., 2013). The electrode was left in place for 5 minute after the injection. The rats were then returned to their home cage and allowed to recuperate for 8 days (see Figure 2.1). This also allowed the FLG necessary time to retrogradely travel to the perikarya of the efferent processes projecting to the VTA (Fahrbach et al., 1986).

2.3.2.2. Experiment 2

Anatomical connections between the mPOA and the VTA were examined using the anterograde tract-tracer biotinylated dextran amine (n = 6; 10000 MW; BDA; Sigma-Aldrich, St. Louis, MO) injected into the mPOA (AP, -0.25 mm; ML -0.5 mm; DV, -8.5 mm). A final set of animals (n = 6) received iontophoretic injections of both FLG into the dorsomedial shell of the NAc (AP, +1.0mm; ML, +1.1mm; DV, -7.0mm) and BDA into the mPOA (same coordinates as above). See figure 2.1 for an illustrated representation of these injections. Surgeries were performed with assistance of a stereotaxic apparatus while animals were still under general anesthesia immediately after the ovariectomy. The skull was exposed via a medial incision and the brain exposed directly above the regions of interest. A glass electrode (opening, ~20 μm diameter) containing the tract tracer was lowered to the predetermined coordinates. The tract-tracer was iontophoretically injected (+7 μA; 7 seconds on, 1 second off for 5 minutes) and was left in place for one minute after the injection and then slowly removed from the brain.

2.3.3. Experimental design

2.3.3.1. Experiment 1

Eight days following injection of FLG, rats that only received FLG (n = 24) were then given an injection of cocaine HCl (10mg/kg in 0.9% saline solution; s.c.; Sigma Aldrich, St. Louis, MO) or vehicle (1mL/kg). They were then returned to their home cage for one hour. A subset of female rats (n = 6) received no treatment after the initial surgery. All rats were sacrificed on day eight following the FLG injection.

2.3.4. Tissue collection

To ensure maximal expression of stimulus-induced c-Fos, animals that received cocaine or vehicle were given a lethal dose of Euthasol (0.3 mL/animal, Virbac Animal Health, Inc., Fort Worth, TX) 1 hr after the injection (Kovács, 1998). After animals were irreversibly anesthetized, the chest cavities were exposed and a blunt-tipped needle perforated the apex of the heart and extended into the aorta via the left ventricle. Before the perfusion began the descending aorta was clamped. Thereafter, 50 mL of 0.1M phosphate buffered saline (PBS) was pumped through the circulatory system followed by 250 mL of 4% paraformaldehyde (PFA) in 0.1M phosphate buffer (PB). The brains were removed and postfixed in 4% PFA for 1 hr, then transferred to a 30% sucrose solution for 24 hrs. The brain was transferred once more into fresh 30% sucrose solution and kept at 4°C until slicing. The brains were sliced in 35μm coronal sections using a freezing microtome (Thermo Fisher Scientific Inc., Waltham, MA), placed into a cryoprotectant solution (30% ethylene glycol, 30% sucrose, 0.00002% sodium azide in 0.1M sodium phosphate buffer), and stored at -20°C until used for immunostaining.

2.3.5. Injection placement

Coronal slices containing the VTA were examined to determine the placement of the FLG injection. The tract-tracer autofluoresces at the emission wavelength of 461nm using an excitation wavelength of 365nm; thus, placement was determined using a filter (Filtersatz 49 DAPI shift free, Zeiss; Oberkochen, Germany) specific to this wavelength on a Zeiss Axioscope microscope (Oberkochen, Germany). Only animals that had the injection site within the VTA were used (BDA only: n = 3; FLG only: n = 4; BDA+FLG: n = 5).

For the animals receiving FLG and/or BDA, sections containing the NAc and the mPOA were selected. Injection placement of FLG in accumbal sections was determined as stated above. Preoptic sections containing BDA were washed in 0.1M PB followed by incubation in avidin-biotin complex (Vector Laboratories, Burlingame, CA) for 1h. After a subsequent wash, the tissue was exposed to a solution containing 0.02% 3,3'-diaminobenzidine (DAB), 2% nickel sulfate, and 0.1% H_20_2 in 0.1M PB for 10min. The chromogen reaction was terminated by extensive washes in 0.1M PB.

2.3.6. Immunohistochemistry and confocal microscopy

Free-floating sections containing the mPOA from animals that received a FLG injection in the VTA were used to determine the hormone receptor phenotype and activational effects of cocaine in preoptotegmental efferents. The protocol for fluorescent immunohistochemistry used for this experiment is previously described in Tobiansky et al. (2013). Each subset of tissue was exposed to different steroid hormone receptor (SSHR) antibodies that are described in table 1. Enhancement steps (i.e., avidin-biotin complex and streptavidin) were always used with the HR antibody and the Fos antibody was always left unenhanced. All sections were mounted and coverslipped using Fluoromount™ aqueous mounting media (Sigma Aldrich, St. Louis, MO). Control

sections for all antibodies omitted the primary antibody, which in all cases resulted in no staining. Colocalization of SSHRs NeuN, or Fos and FLG was quantified in 2 bilateral sections of the mPOA (rostral and central) for each animal using National Institutes of Health freeware ImageJ (version 1.47v). Images used to quantify colocalization within each subregion of the mPOA covered a 1.6mm^2 area and were captured using a Zeiss Axio Scope.A1 microscope and AxioVision software (Oberkochen, Germany).

In the second set of experiments immunofluorescence was employed to examine colocalization of FG tract-tracer, GABA, and DA D_2 receptor (D_2R) in the mPOA, or BDA, the nuclear protein DAPI, and VGAT in the VTA. Visualization of GABA and D_2R were obtained after blocking sections for 10 min with 1% H_2O_2 and incubating the tissue overnight in blocking solution with a primary antibody recognizing GABA raised in mouse (1:16,000; Fluorochrome, LLC, Denver, CO). Sections were then exposed for 60 min to biotin-conjugated goat anti-mouse IgG (1:200 in incubation solution; Vector Laboratories, INS, Burlingame, CA) and for 60 min to an avidin-biotin complex (1:1000 in 0.1M PB; ABC-elite; Vector Laboratories, Burlingame, CA). Thereafter the tissue was incubated in a solution containing biotinylated tyramide (1:1000; in 0.1M PB; Perkin Elmer, Waltham, MA) for 10 minutes. An Alexa Fluor 488-tagged streptavidin (1:400 in 0.1M PB; Life Technologies, Grand Island, NY) was then introduced for one hour. The second fluorescence complex was visualized with the same procedure described above but without the enhancement steps. An anti-rabbit D_2R antibody (1:800; EMD Millipore, Billerica, MA) was introduced followed by an Alexa Fluor 555-tagged goat anti-rabbit secondary antibody (1:200; Life Technology Corporation, Carlsbad, CA). The anterograde tract-tracer BDA was visualized in coronal sections containing the VTA using the same enhancement steps. The tissue was incubated in Alexa Fluor 488-tagged streptavidin for 30 minutes. Primary antibodies used were rabbit anti-VGAT (1:2400;

EMD Millipore, Billerica, MA) followed by secondary antibodies (Alexa Fluor 555-tagged goat anti-rabbit, 1:100, Life Technology Corporation, Carlsbad, CA). Sections were mounted and coverslipped. Control sections for all antibodies included omission of the primary antibody, which resulted in no staining.

Finally, immunofluorescent staining for BDA and tyrosine hydroxylase (TH) on tissue containing the VTA of animals that received an injection of BDA in the mPOA and FLG in the NAcS followed the same protocol as described in Tobiansky et al. (2013). Information about the TH antibody is provided in Table 1. The tissue was then mounted and coverslipped.

Z-stacks (15μm thickness; 10 slices per stack, 1.25 NA × 40 oil immersion lens, optical zoom 2) of VTA sections containing FLG, BDA and TH were captured using a confocal laser-scanning microscope (TCS SP2; Leica Microsystems, Mannheim, Germany). Sequential scanning with excitation wavelengths of 395, 488 and 555 nm and a line averaging value of 4 was used during image acquisition. Number of FLG-positive cells, TH-ir cells, and apposition (as determined by synaptic boutton proximity to perikarya at 100 nm or less; Cooke & Woolley, 2005) of mPOA efferents (BDA-containing processes) to FLG-positive and TH-ir cells in the VTA were manually quantified using ImageJ.

2.3.7. Statistical analyses

All data were analyzed using PASW statistical package (18th Ed). A repeated measures ANOVA was used to determine within animal differences between subregions of FLG concentrations in the mPOA, with a Tukey multiple comparison test to determine specific between-subregion differences. A univariate ANOVA was used to analyze all data unless assumptions were not met, in which case, a Kruskall-Wallis nonparametric

test and Mann-Whitney U (for pairwise comparisons) were used. For normally distributed data, Tukey post-hoc tests were used to determine between group differences only if there were statistically significant effects. Data comparing Fos-immunoreactivity in mPOA-VTA efferents were weighted by multiplying the percentage of colocalization within FLG+ cells by $1/\sqrt{}$(mean number of FLG+ in the animal; i.e., %colocalization*$(1/\sqrt{n})$) due to the variance of FLG+ cells in the mPOA per animal before any statistical comparisons were made. Finally, we performed a linear regression analysis to confirm that the number of FLG-positive cells was not directly related to the number of Fos-positive cells in the mPOA. Statistical significance was set at $\alpha = 0.05$ for all analyses.

Antigen	Host Animal	Mono vs Polyclonal	Enhancement	Company	Secondary	Fluorophore	Concentration
NeuN	Mouse	Mono	Yes	Millipore	Goat anti-rabbit	Alexa 555	1:1000
Fos	Rabbit	Poly	No	Santa Cruz Biotechnology	Goat anti-rabbit	Alexa 555	1:500
Fos	Mouse	Mono	No	Santa Cruz Biotechnology	Goat anti-mouse	Alexa 555	1:500
ERα	Rabbit	Mono	Yes	Millipore	Goat anti-rabbit	Alexa 488	1:800
PR	Mouse	Mono	Yes	Thermo Scientific	Goat anti-mouse	Alexa 488	1:1000
GPER (GPR30)	Rabbit	Poly	Yes	Santa Cruz Biotechnology	Goat anti-rabbit	N/A	1:50
PGRMC1	Rabbit	Poly	Yes	Santa Cruz Biotechnology	Goat anti-rabbit	Alexa 488	1:100
TH	Sheep	Poly	No	Millipore	Donkey anti-sheep	Alexa 555	1:500
VGAT	Rabbit	Poly	No	Millipore	Goat anti-rabbit	Alexa 555	1:2400

Table 1. Antibodies used and relevant information for immunohistochemical staining

2.4. RESULTS

2.4.1. Experiment 1

2.4.1.1. Subregional distribution of preoptotegmental perikarya

Rostral and central mPOA (mPOAr and mPOAc, respectively) contain the majority of the perikarya that send projections to the VTA (Figure 2.1). Using a Kruskal-Wallis one-way ANOVA, we found a statistically significant main effect of region within animals [$H_{(2)} = 84.69$; $p < 0.0001$]. In particular, the central region (104.2 ± 8.19) had significantly more FLG-positive cells than the rostral region (47.78 ± 3.65; $p < 0.01$), which in turn had significantly more FLG-positive perikarya than the caudal region (2.22 ± 0.27; $p < 0.001$). Because the rostral and central subregions of the mPOA contained the majority of cells projecting to the VTA, all subsequent experiments that explored the colocalization of tract-tracers or proteins of interest focused on only these subregions.

2.4.1.2. Differences in connectivity to subregions of the ventral tegmental area

Injections in DA-rich subregions [i.e., parabrachial nucleus (PBP) and paranigral nucleus (PN)] and GABA-rich subregions [i.e., ventral tegmental tail (VTT), now commonly referred to as the rostromedial tegmental nucleus (RMTg)] showed higher connectivity to the mPOAc than the mPOAr ($F_{(1,64)}= 47.21$, $p < 0.001$; see Figure 2.1. F**),** regardless of the placement of FLG in the VTA. These results give insight into the potential interactions between the mPOA and the VTA and possible downstream effects, but, since there were no rostral-caudal differences, these comparisons were set aside when analyzing colocalization of FLG+ cells in the mPOA.

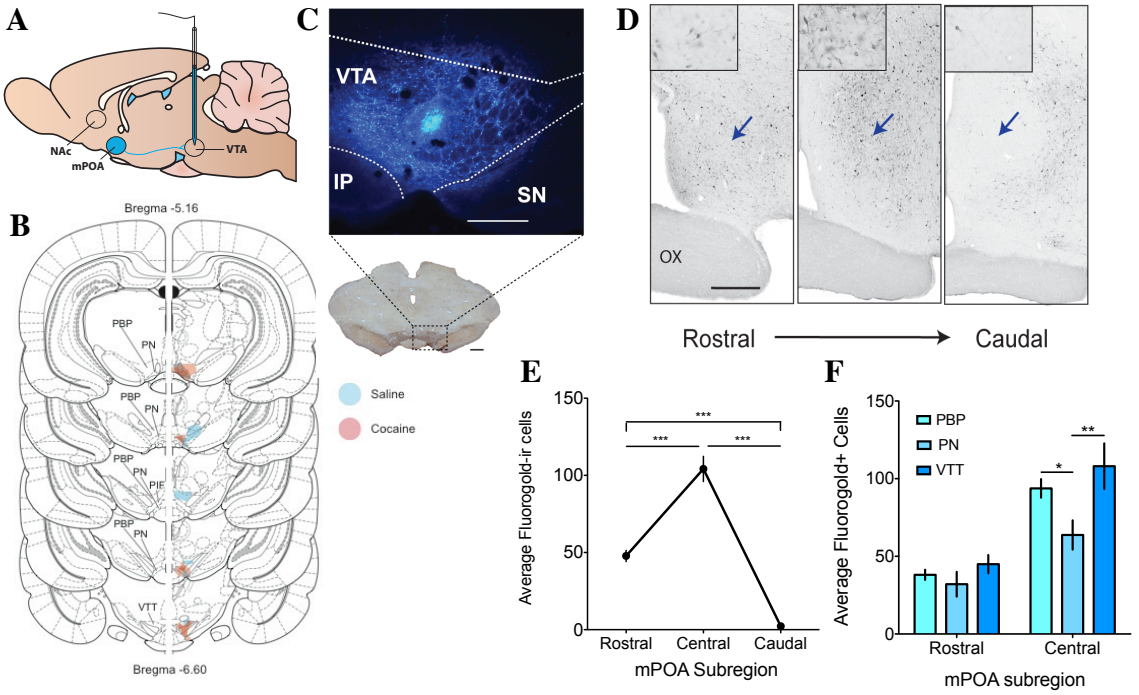

Figure 2.1. Distribution of preoptotegmental efferents. (A) A cartoon image of the Fluorogold iontophoretic injection in the VTA. (B) Injection placement of Fluorogold in the VTA by experimental group (saline: n = 7; cocaine: n = 8) Adapted from Paxinos and Watson (2007) brain atlas. (C) A representative photomicrograph of the FLG injection in the VTA. Scale bar, 500 µm; IP, SN, substantia nigra. (D) Representative distribution of Fluorogold in the mPOA in a rostral to caudal layout. Scale bar, 500 µm. OX, optic chiasm; AC, anterior commisure; 3V, Third ventricle. (E) Average number of Fluorogold positive cells rostral, central, and caudal mPOA. (F) Average number of Fluorogold-positive cells in the rostral and central mPOA as a function of subregional injection placement in the VTA (PBP: parabrachial pigmented nucleus; PN: paranigral nucleus; VTT: tail of the VTA). (F) Subregional distribution within the mPOA of average number of cells that project from the mPOA to the VTA. Values are expressed as mean ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

2.4.1.3. Sex steroid hormone-sensitive cells in preoptotegmental perikarya

An overview of SSHRs concentrations present in the preoptotegmental efferents is presented in Figure 2.2. 97.80% ± 1.5% of mPOA-VTA efferents were immunopositive for NeuN (a neuronal marker; n = 4). Membrane-associated hormone

receptors [i.e., GPER and progesterone receptor membrane component 1 (PGRMC1)] were present on average in 28% of the efferents (28.46% ± 4.81% in the mPOAr and 58.38% ± 5.00% in the mPOAc for GPER and 60.85% ± 3.97% in the mPOAr and 86.56% ± 1.26% for PGRMC1) in the mPOAc. Colocalization of traditional nuclear SSHRs were seen in disparate quantities, with ERα (6.52% ± 0.73% in the mPOAr and 55.08% ± 5.04% in the mPOAc) being the higher of the two, and then a near absence of PR (<1% in both the mPOAr and mPOAc). There was a significant main effect of expression of SSHR levels in FLG+ cells ($F_{(4,216)} = 186.30$ $p < 0.0001$). A Tukey multiple comparison test determined that most SSHRs were seen in significantly different concentrations from one another within the same subregions of the mPOA (see Figure 2.2).

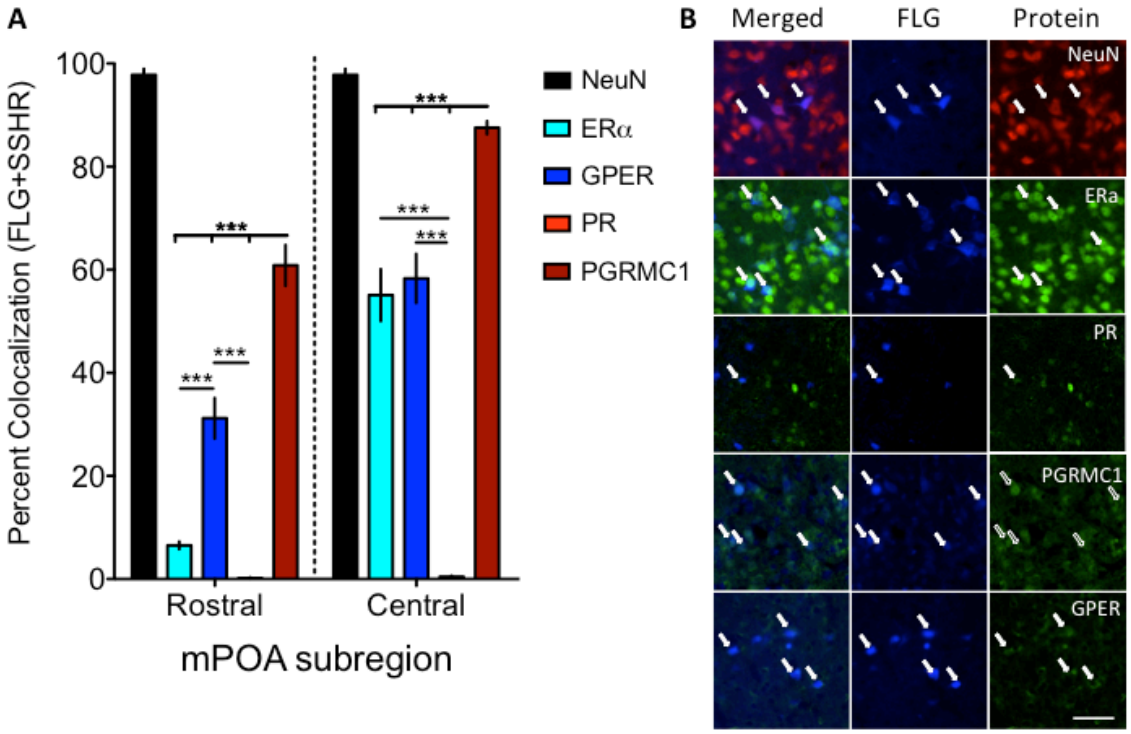

Figure 2.2. Sex steroid hormone receptor concentrations in preoptotegmental efferents. (A) Almost all Fluorogold-positive (FLG+, blue) cells in the VTA colocalize with the neuronal marker NeuN. Both membrane-associated hormone receptors (GPER and PGRMC1) show a consistently higher colocalization with FLG+ than traditional nuclear receptors (PR and ERα). PR is almost nonexistent in preoptotegmental efferents while ERα colocalizes with FLG+ cells in the central mPOA in high quantities. Values are expressed as means ± SEM. *** $p < 0.001$. (B) Representative photomicrographs of colocalization of NeuN (red) or sex steroid hormone receptors (green) and FLG (blue) in the mPOA. Arrows point to cells demonstrating colocalization. Scale bar, 50μm.

2.4.1.4. Cocaine-induced Fos-immunoreactivity in preoptotegmental perikarya

While the data presented above suggest that these efferents are sensitive to both E_2 and P_4, those analyses do not give us insight into how cocaine may influence the activity of these efferents. As such, analysis of percent colocalization of Fos-ir in FLG+ perikarya exhibited a trend towards a cocaine-mediated increase in Fos-ir in rostral

mPOA-VTA efferents [$F_{(1,19)} = 5.168$, $p = 0.08$]. A regression analysis confirmed that the presence of FLG in the mPOA did not predict Fos-ir within the mPOA (mPOAr: $r^2 = 0.04$, $p = 0.499$; mPOAc: $r^2 = 0.06$; $p = 0.407$). See figure 2.3 for graphical representations of the data.

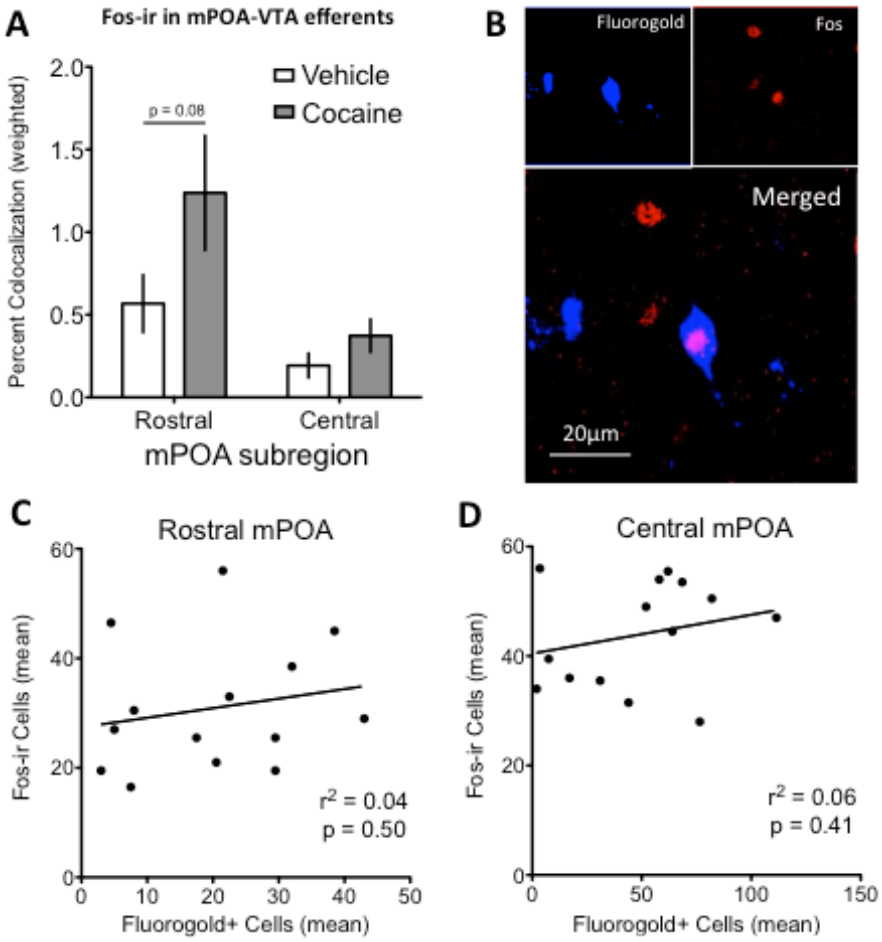

Figure 2.3. Cocaine enhances activity in preoptotegmental perikarya. (A) Graphical representation of weighted percent colocalization of Fos-immunopositive cells and FLG in the rostral and central mPOA after acute cocaine administration. (B) Representative photomicrographs of Fos-immunoreactive cells (red) colocalized with FLG-positive cells (blue). (C, D) Scatter plots with a fitted regression line demonstrating that the presence of FLG in the perikarya in the rostral or central mPOA does not predict the presence of Fos-ir.

2.4.2. Experiment 2

2.4.2.1. Inhibitory and DA-sensitive preoptotegmental efferents

After injecting FLG in the VTA (n = 4), we examined FLG-positive, GABA-ir, and D_2R-ir cells in the rostral mPOA and discovered that a large percent of mPOA-VTA

efferents contained GABA (67.6 ± 6.5%) and D_2R (76.3 ± 6.5%), whereas 58.3% ± 7.3% contained both GABA and D_2R (Figure 2.4.A.). To further validate the apparent GABAergic profile of mPOA-VTA efferents, we injected the anterograde tract tracer BDA in the mPOA followed by analysis of BDA and the vesicular transporter protein for GABA (VGAT) in the VTA. Qualitative analyses revealed that many of these fibers contained VGAT (Figure 2.4.B.), further demonstrating that many mPOA-VTA efferents are GABAergic. We note that the neurotransmitter content associated with mPOA-VTA efferents was heretofore unknown, so our initial analysis of exclusively GABA-containing neurons does not discount possible influences of other neurotransmitters. Nonetheless, our results indicate that the rostral mPOA modulates mesolimbic activity via GABAergic output, which may then regulate DA release in the NAc. Moreover, this mPOA output, itself, is receptive to DA activity, as evidenced by DA receptors colocalized with efferent neurons.

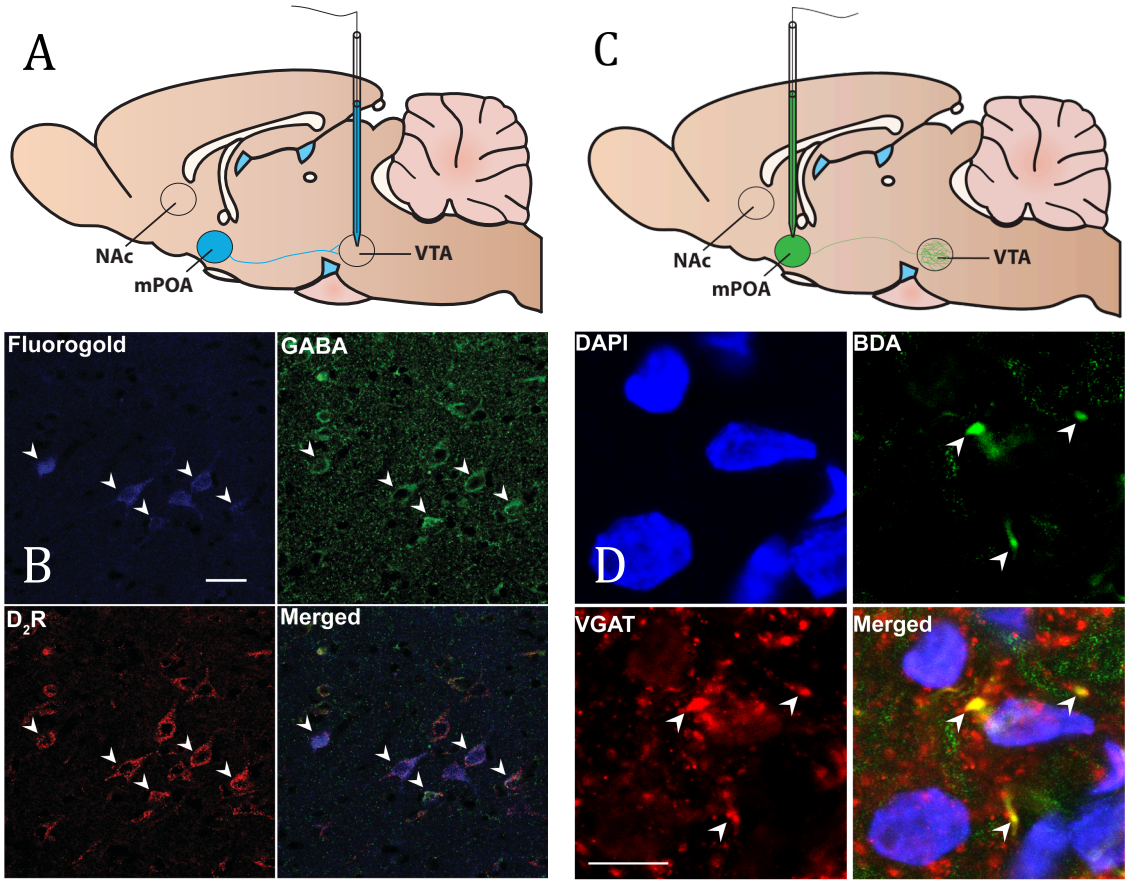

Figure 2.4. mPOA-VTA efferents contain GABA and DA receptors. (A-B) Cartoon representations of a sagittal section of the rat brain depicting (A) a Fluorogold injection in the VTA and (B) a BDA injection in the mPOA. (C) Confocal photomicrograph demonstrating colocalization of mPOA-VTA efferents (Fluorogold, blue), GABA (green), and D_2R (red). Analyses of mPOA-VTA efferents revealed that 67.6 ± 6.5% contained GABA, 76.3 ± 6.5% contained D_2R, and 58.3% ± 7.3% contained both GABA and D_2R, indicated by arrows. (D) Following injection of the anterograde tract tracer biotinylated dextran amine (BDA) into the mPOA, qualitative analyses of BDA (green) and the vesicular transporter protein for GABA (VGAT, red) in the VTA revealed that most BDA-positive fibers contained VGAT, further validating the GABAergic profile of this pathway. Also shown here is DAPI nuclear labeling in blue; scale bars are 10 μm.

2.4.2.2. Preoptotegmentostriatal DAergic circuitry

Cells in the mPOA project to the VTA, but the specific downstream targets of these efferent fibers have not been definitively identified. Regions downstream of these connections have been heretofore undetermined. Consistent with previous studies (Margolis, Lock, Hjelmstad, & Fields, 2006; Nair-Roberts et al., 2008), the majority of cells in the VTA that project to the NAcS were DAergic (i.e., TH-ir; 77.3%). Of all of the VTA perikarya projecting to the NAcS 45.4% were apposed by mPOA efferents, and 41.3% of the VTA cells apposed by mPOA efferents contained TH-ir. A moderate percentage (45.6%) of the DArgic cells, regardless of the area they project to, in the VTA were apposed by fibers originating from cells in the mPOA. All cells and apposition percentages were quantified ipsilaterally to the site of tract tracer injections. See figure 2.5 for a summary of the results.

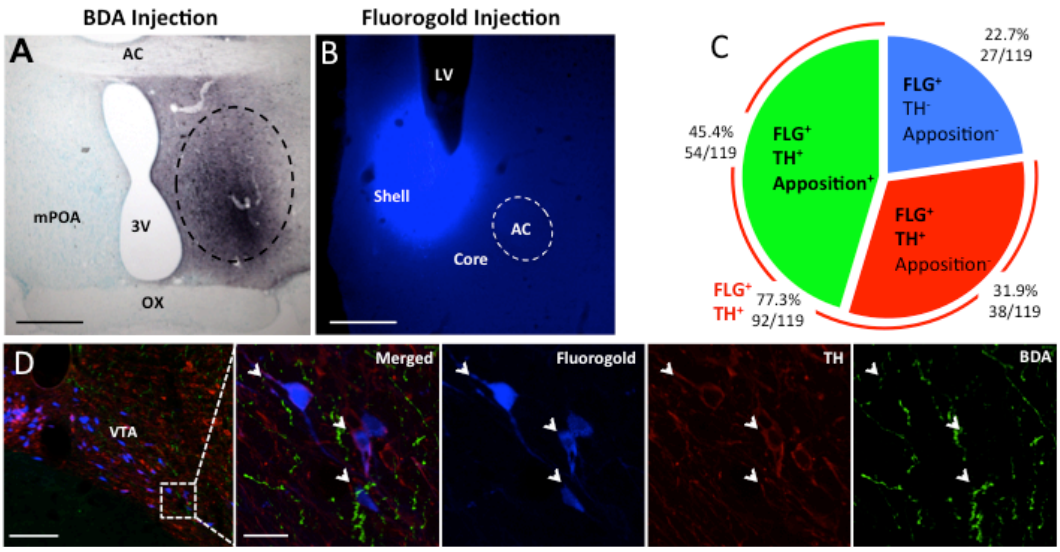

Figure 2.5. mPOA efferents appose VTA DAergic tegmentostriatal perikarya. (A) A representative photomicrograph of placement of biotinylated dextran amine, an anterograde tract-tracer, in the mPOA. Scale bar, 500µm (B) A representative photomicrograph of Fluorogold placement, a retrograde tract-tracer in the shell of the nucleus accumbens. Scale bar, 500µm (C) A pie chart describing the percentage of cells of all cells sampled in the VTA apposed or unapposed by BDA-positive processes. (D) Representative photomicrographs of coronal sections of the VTA containing Fluorogold (blue), TH (red), and BDA (green). Arrows represent points of apposition. Scale bars, 200µm and 20µm from left to right. AC, anterior commisure; 3V, 3rd ventricle; OX, optic chiasm; LV, lateral ventricle; TH, tyrosine hydroxylase; BDA, biotinylated dextran amine.

2.5. Discussion

Previous literature has demonstrated that sex steroid hormones play an essential role in the acquisition, maintenance and reinstatement of responding for drugs of addiction (Becker, Molenda, & Hummer, 2001; Becker & Hu, 2008; Chin et al., 2002; Festa & Quiñones-Jenab, 2004; Niyomchai, Akhavan, et al., 2006; Russo, Festa, et al., 2003). The research presented here elucidates one of the mechanisms by which SSHs

may influence cocaine-mediate neural activity. In brief, we were able to replicate findings that the mPOA sends projections to the VTA in a subregion-specific manner with most of the projections originating from the rostral and mPOAc (Fahrbach et al., 1986; Struthers, 2001; Swanson et al., 1987; Tobiansky et al., 2013). Furthermore, general activity of preoptotegmental cells as indicated by Fos-ir was increased, albeit a trend, in preoptotegmental perikarya as a function of cocaine treatment. We also found that SSHRs are present in a large portion of the mPOA perikarya that project to VTA. Moderate to high percentages of both rostral and central mPOA efferent cells also contained G protein-coupled receptors (GPCRs) specific to estrogens (i.e., GPER) and progestins (i.e., PGRMC1), but ERα was the only classic nuclear receptor expressed in a high percentage of efferents, and this was only the case in the central mPOA cells. The relative abundance of SSHRs in different efferent mPOA cell populations suggests that SSHs in the mPOA may be important for the rapid modulation of VTA excitability. Cocaine moderately influenced the activity of mPOA neurons projecting to the VTA, particularly in ERα-ir cells. Additionally, these mPOA neurons may modulate cocaine-induced activity in the mesolimbic pathway, as anterograde and retrograde tract-tracing confirms that preoptotegmental efferents also appose DAergic cells in the VTA that then project to the NAcS. Because the VTA-to-NAcS circuitry is one of the principal mediators of natural and drug-mediated reward (see Hull, 2011; and Koob & Volkow, 2010 for review), these connections may provide a mechanism through which SSHs can influence response to cocaine.

It is understood that GABA coming into the VTA arises locally in the VTA and in the rostromedial tegmental nucleus. Here, however, we showed, for the first time, that at least a portion of the GABA entering the VTA originates in the mPOA. This link may help explain why lesions of the mPOA augmented cocaine-induced activity in the NAc

and the ensuing cocaine-reward response as the lesions effectively removed a source of GABAergic input into the VTA, which allowed for the disinhibition of its output into the NAc. There are many potential outcomes of decreased activity of the VTA, which contains functionally and cytoarchitecturally different subregions. If the cells of the mPOA project to the DAergic portion of the rostral VTA, activity in the mPOA might result in a net decrease in DA output onto medium spiny neurons in the NAcS. On the other hand, if these cells are projecting to the GABAergic tail of the VTA (see Ikemoto, 2007 for review), mPOA activation might disinhibit DAergic VTA neurons. This study found that mPOA projections to the VTA appose 45.4% of DAergic cells in the VTA that then project to the NAcS. Furthermore, injections localized in both DA-rich and GABA-rich areas of the VTA showed similar connectivity to the mPOA. The complexity of the system is understated and it is likely the ratio of DAergic-to-GABAergic cells receiving input from preoptotegmental neurons that assists determining the output of the VTA. Regardless, these findings point to a modulatory role for the mPOA in cocaine-induced mesolimbic activity, through GABAergic connections coming primarily from its rostral region.

The existence of this connection should not be surprising, given that the mPOA regulates naturally rewarding sexual and maternal behaviors (Stolzenberg & Numan, 2011). The mPOA also contains robust connections with the mesolimbic system (Simerly & Swanson, 1988). The importance of the mPOA for mating is evidenced in several studies showing that lesions significantly impair both male and female sexual behavior, whereas stimulation facilitates both consummatory and appetitive aspects of behavior (Guarraci & Clark, 2006; Hull & Dominguez, 2007; Yang & Clements, 2000). Conversely, neuronal activity in the mPOA increases with mating and in the presence of sexually relevant stimuli (Hull & Dominguez, 2007; Pfaus & Heeb, 1997). Moreover,

with regard to the mPOA and maternal behavior, in pre- and postpartum rat dams, lesions of this area severely disrupt maternal behaviors. Finally, disruption of connections between the mPOA and VTA attenuate retrieval behavior (Numan et al., 2009) and impede operant responses to obtain access to pups (Lee et al., 1999). Together, these data have led to the proposal that mPOA efferents activate VTA DA input to the NAc (Numan and Stolzenberg, 2009).

Initially, our findings appear to counter this proposed model, as we found that a large number of cells projecting from the mPOA to the VTA are GABA-producing neurons. However, a more recent view of the mPOA and its function suggests varying roles for different subregions within the mPOA in regulating different aspects of behaviors (Balthazart & Ball, 2007). In fact, large bilateral lesions of the mPOA are required to impact behavior, whereas smaller or unilateral lesions have little, if any, impact (Hull & Dominguez, 2007; Numan et al., 2009). Our experiments focused on the rostral mPOA, where we discovered that cocaine increases activity in preoptotegmental perikarya, and that 68% of mPOA-VTA efferents were GABA-producing neurons, which suggests that perikarya in the rostral mPOA may further inhibit the VTA after cocaine administration.

The second goal of this study was to determine the SSHR phenotype of preoptotegmental perikarya. This goal stems from ample evidence that SSHs modulate the salience of artificial and natural rewards, in part by influencing phasic DA release in the NAc (Bazzett & Becker, 1994; Hull, 2011; Jackson et al., 2006; Robinson et al., 1982). Given the paucity of SSHRs in the mesolimbic circuitry (Intlekofer & Petersen, 2011; Österlund, 1998; Simerly et al., 1990), this study tried to elucidate other neural nodes that are sensitive to SSHs and influence the mesolimbic system. Here we show, through immunohistochemical staining, that preoptotegmental efferents are highly

sensitive to progestogens and estrogens. These steroid-sensitive connections may be one way in which hormones modulate DA release in the NAc. Due to the variety of ways in which SSHs influence the excitability and protein expression profile of neurons containing receptors specific to these hormones (D. P. Edwards et al., 1995; Gordon et al., 2009; Hull et al., 1999; Panzica et al., 2011; Tsuda et al., 2011), it is difficult to determine precisely how these projections may influence the DAergic output of the VTA without further study. Yet, we found that the mPOA-to-VTA efferents contained more membrane-associated receptors than traditional nuclear receptors. These membrane-bound receptors are fundamental to the rapid electrophysiological responses of neurons in response to SSHs (Dennis et al., 2009; Graves, Hayes, Fan, & Curtis, 2011; Prossnitz & Maggiolini, 2009; P. Thomas, 2012). Furthermore, interactions between psychostimulants and ovarian hormones are likely mediated, at least in part, by these membrane receptors (Alyea et al., 2008).

The estrogen-responsive receptor GPER, generally localized in the plasma membrane (Funakoshi, 2006) and the endoplasmic reticulum (Langer et al., 2010), is an ideal candidate for rapid modulation of neuronal activity in the mPOA. In vitro activation of this seven-transmembrane protein facilitates Ca^{2+} (Kuo, Hamid, Bondar, Prossnitz, & Micevych, 2010) and cyclic AMP (Filardo et al., 2007) signaling. Roepke and colleagues (2009) found that E_2 activation of GPER leads to in increase in phospholipase C, which in turn leads to an increase in the catalytic activity of protein kinase A and protein kinase C delta (PKC δ). This increase in PKC δ ultimately results in inhibition of $GABA_B$ receptors and therefore greater neuronal excitability. Because most mPOA projections to the VTA are GABAergic, elevated neuronal excitability and release of internal stores of Ca^{2+}, which increases the probability of the vesicular release of GABA (Carrasco, Jaimovich, Kemmerling, & Hidalgo, 2004; Kelm, Criswell, & Breese, 2011), might

ultimately lead to downstream disinhibition of the VTA if these cells interact directly with DAergic neurons.

Much like GPER, the progestogen-specific membrane receptor PGRMC1 is also responsible for rapid mediation of neuronal excitability by P_4 (Bali et al., 2012; see Petersen et al., 2013 for review). Of all the SSHRs examined, we found that PGRMC1 has the highest colocalization levels in preoptotegmental efferents. PGRMC1 regulates intracellular levels of Ca^{2+} (Bashour & Wray, 2012; Vilner & Bowen, 2000) and also mediates cell signaling through protein kinase G (PKG; Peluso, Liu, & Romak, 2007; Peluso & Pappalardo, 2004). Administration of a PGRMC1 antagonist attenuates cellular inhibition by P_4 (Peluso and Pappalardo, 2004; Peluso et al., 2007) possibly via its influence on PKG, which has been shown to mediate P_4 induced Ca^{2+} flux in gonadotropin-releasing hormone (GnRH) neurons (Bashour & Wray, 2012). PGRMC1 may also influence cellular activity through its ability to mediate steroid synthesis. In particular, PGRMC1 activates Cyp19 aromatase, which is required for E_2 synthesis (Ahmed, Chamberlain, & Craven, 2012); implications of increased E_2 levels in the mPOA are discussed below. Thus, PGRMC1 may be involved in influencing the activity of preoptotegmental efferents in two ways. The first is a general inhibition of activity thus releasing inhibition on VTA DA signaling. Considering the high concentrations of PGRMC1 mRNA in the mPOA (Intlekofer & Petersen, 2011a; Krebs et al., 2000), and that systemic P_4 increases DA release in the mPOA (Matuszewich et al., 2000), P_4 may be acting through PGRMC1 to inhibit GABAergic preoptotegmental efferents to the VTA, ultimately increase DA release in the NAc. Secondly, an increase local E_2 synthesis may influence E_2-specific pathways via GPER, as discussed above, and nuclear ERs.

While the abundance of ERα in the mPOA has been clearly established, its role in modulating the activity of preoptotegmental efferents is much more ambiguous.

Behavioral studies suggest that ERα in the mPOA is necessary for the expression of maternal and sex behavior. For example, a siRNA-mediated down-regulation of ERα in the mPOA of female mice abolished maternal care (Ribeiro et al., 2012). Although that study did not explore neuroanatomical connections, it is possible that ERα downregulation in preoptotegmental efferents at least partially explains the results. There are several cellular mechanisms through which ERα influences the activity of preoptotegmental efferents. Previous studies have shown that ERα is present in the nucleus and the plasma membrane and can have both genomic and non-genomic actions (Kuo et al., 2010; McEwen & Alves, 1999; see Vasudevan & Pfaff, 2008 for review). Many, if not most, of the ERα-ir cells in this study displayed both nuclear staining and a fainter membrane staining (see figure 2.2). This implies that E_2 in the mPOA may modulate neuronal activity via protein translation and also via more rapid, intermediate messengers. Chronic E_2, as was used in this study, increases transcription of the 65kDa form of the enzyme glutamic acid decarboxylase (GAD_{65}; McCarthy, Kaufman, Brooks, Pfaff, & Schwartz-Giblin, 1995), which is responsible for local GABA synthesis in synapses (Herbison & Fenelon, 1995). Taken together, this suggests that E_2 in the mPOA may increase production of GABA, potentially making it a robust inhibitory node regulating the VTA. At the same time, E_2 may influence cellular activity through membrane bound ERα and its downstream effects on the PI3K-Akt signaling pathway (Cardona-Gomez, Mendez, & Garcia-Segura, 2002; Mannella & Brinton, 2006) or rapid ERK phosphorylation (Abrahám, Todman, Korach, & Herbison, 2004). Finally, ERα may also influence cellular signaling in preoptotegmental efferents through a ligand-independent phosphorylation mechanism. This process allows the receptors to homodimerize, translocate to the nucleus, and bind to the DNA at estrogen response

elements (ERE) in promoter regions, producing similar effects to the ligand-bound state (see Heldring et al., 2007 for review).

In all, we found that preoptotegmental efferents show differential expression of both nuclear hormone receptors and membrane-associated hormone receptors, particularly in the mPOAc. PGRMC1, a membrane receptor located in the endoplasmic reticulum, was found in the largest proportion of mPOA-to-VTA efferents. The two E_2-responsive receptors, GPER and ERα, were found in over half of the efferents. On the other hand, the P_4 nuclear receptor PR was almost entirely absent from all efferents and likely do not play a large role in modulating neuronal activity in these cells. The exact roles of the aforementioned SSHRs in preoptotegmental efferents still remain to be elucidated, but may provide some insight into possible actions.

2.6. Summary and conclusions

The results shown here suggest that preoptotegmental efferents may play a role in modulating the activity of the VTA, and thus the mesolimbic circuitry, following cocaine administration. P_4's widely accepted role in attenuating cocaine-mediated conditioned approach behavior (Lynch et al., 2000; Niyomchai et al., 2005; Quiñones-Jenab & Jenab, 2010; Russo, Festa, et al., 2003) or reward (Evans, 2007; Reed et al., 2011; White et al., 2002) may be due, at least in part, to its actions in the mPOA. The high proportions of mPOA-to-VTA efferents containing membrane-associated hormone receptors and ERα suggest that P_4 and E_2 levels in the mPOA may modulate conditioned approach behavior. Indeed, the cocaine-mediated enhancement of Fos-ir in preoptotegmental cells demonstrates that the mPOA might impact activity of the mesolimbic DA system. Though further investigation of SSHR influences on preoptotegmental projections is certainly needed, the results of this study clearly point to a role for the mPOA as a neural

node through which SSHs can alter drug-induced mesolimbic system activity and behaviors.

Chapter 3: Effects of mPOA lesions on cocaine-mediated brain activity and striatal dopamine release[2]

3.1. ABSTRACT

The medial preoptic area (mPOA) is involved in the regulation and expression of naturally rewarding behaviors in both sexes. In females, it mediates maternal behaviors, and appetitive and consummatory sexual behavior. Furthermore the mPOA sends extensive inhibitory projections to the ventral tegmental area (VTA), potentially influencing the activity of the mesolimbic dopamine (DA) system. Acute cocaine, a potent psychostimulant, increases activity of DAergic neurons in the VTA and enhances DA release in the nucleus accumbens (NAc). Previous evidence has indicated that the mPOA might also influence cocaine-mediated activity in the mesolimbic DA system. In this study we sought to determine whether the mPOA influences activity and DA release in the NAc in response to cocaine. In the first experiment, mPOA-lesioned and intact female rats were given an acute injection of cocaine or vehicle. Fos-immunoreactivity, used as a marker for cellular activity, was assessed *ex vivo* in regions associated with the mesolimbic DA system. In the second study, microinjections of neurotoxic levels of N-methyl-D-aspartate (NMDA) or vehicle were infused into the mPOA of female rats. Following complete recovery, a microdialysis probe was placed into the shell/core region of the NAc of freely moving rats. Dialysate samples were collected at baseline levels, after a saline injection, and after a cocaine injection (10mg/kg, i.p.; 6 samples) in 15-minute bins. High performance liquid chromatography was used to analyze DA and 5-hydroxytryptamine (5-HT) levels in dialysate samples. Results in the first experiment

[2] Portions of text in this section are excerpted from Tobiansky DJ, Roma PG, Hattori T, Will RG, Nutsch VL, Dominguez JM, Behavioral Neuroscience 127(2), 293-302. PGR assisted in establishing the conditioned place preference paradigm in our lab. TH, RGW, VLN and JMD were involved in edits and assisted in surgery.

demonstrated that mPOA radiofrequency lesions enhanced cocaine-induced Fos-immunoreactivity in the NAc. In the second experiment, dialysate samples contained comparable baseline and saline-mediated levels of monoamines in both intact and lesioned animals. In contrast, there was a significant cocaine-mediated increase in both analytes. Importantly, the lesion group demonstrated enhanced DA release compared to the sham lesion group. These results suggest that the mPOA may play a role as an inhibitory node that directly influences DA release in in the mesolimbic reward system in response to acute cocaine. These results underline the need to recognize the mPOA as an integral region involved in the processing of reward-related stimuli, both natural and artificial.

3.2. INTRODUCTION

The medial preoptic area (mPOA) plays an essential role in the expression of naturally reinforcing behavior in both males and females. In males, ablation or inhibition of the mPOA causes significant deficits in sex behavior (Been & Petrulis, 2010; Hull & Dominguez, 2007; Liu et al., 1997; Powers et al., 1987)). In females, damage to the mPOA attenuates maternal behaviors (Numan and Insel, 2003; Numan et al., 2005; Lee, Clancy and Fleming, 1999; Arrati et al., 2006; Jacobson et al., 1980) and impacts the expression of sex behaviors (Hoshina et al., 2004; Sokuma, 2008). For example, Yang and Clements (2000) found that lesions of the mPOA increased a female rats latency to return to the male during a bout of paced mating. In contrast, Rodriguez-Sierra and Terasawa (1979) discovered that electrolytic lesions of the mPOA enhanced lordosis behavior in female guinea pigs. Finally, natural reinforcers also enhance activity in the mPOA of female rats as inferred by the expression of the immediate early gene Fos (Pfaus & Heeb, 1997; Pfaus, Marcangione, Smith, Manitt, & Abillamaa, 1996; Stack et

al., 2002; Tsuneoka et al., 2013). These findings suggest that the mPOA is involved in the expression of several aspects of naturally reinforcing behaviors and, at least in females, may differentially regulate appetitive versus consummatory behaviors.

Recent research suggests that the mPOA in female rats may also be involved in regulating the reinforcing aspects of psychostimulants. Psychostimulants such as cocaine are thought to have their reinforcing effects through the mesolimbic dopamine (DA) reward system (Berridge & Arnsten, 2013; Ikemoto & Bonci, 2014; Ikemoto, 2007; Wise, 2002). This system, which consists of DArgic cell bodies in the VTA that project to the nucleus accumbens (NAc) in the forebrain, is responsive to both natural and artificial reinforcers (Kelley & Berridge, 2002; J. Matsumoto et al., 2012; Olsen, 2011). Cocaine and other cocaine-like stimulants influence DA release at the terminals of these cells by inhibiting the DA transporter (DAT) protein, thus increasing the available DA in the synaptic cleft (Kuhar et al., 1991; Ritz et al., 1987; Wilcox et al., 1999)). The mPOA has reciprocal connections with this pathway via DAergic VTA neurons (Ikemoto & Bonci, 2014; Miller & Lonstein, 2009; Simerly & Swanson, 1986, 1988; Tobiansky et al., 2013), but does not send efferents directly to the NAc (Simerly & Swanson, 1988). This indirect circuit likely mediates monoamine release and subsequent activity in NAc system prompted by naturally reinforcing stimuli (Carelli, 2002; Carlezon & Thomas, 2009; Olsen, 2011). Furthermore, cocaine increases activity in the mPOA (Tobiansky et al., 2013), but not as strongly as activity induced by pups in postpartum dam rats (Mattson & Morrell, 2005). Considering the high density of DAergic (Miller & Lonstein, 2009) and noradrenergic (Smith et al., 2012) inputs into the mPOA and presence of D_2-like receptors (Khan et al., 1998; Tobiansky et al., 2013) it is not surprising that cocaine has an effect. Yet, how cocaine-induced activity in the mPOA influences DA release in the mesolimbic reward system is not known.

The aforementioned studies suggest that the mPOA may modulate cocaine-induced neurotransmitter release in the NAc, particularly in female rats. The experiments reported below were performed to determine whether the mPOA influences activity in the mesolimbic DA system, and accumbal monoamine transmission and behaviors associated with cocaine. We hypothesized that the mPOA would enhance activity in the mesolimbic DA system and DA transmission in the NAc, but will not influence cocaine-mediate locomotion or 5-HT release. This would suggest that the mPOA is directly involved in regulating the reinforcing aspects of a psychostimulant via the mesolimbic system and is not a consequence of activity in motoric or serotonergic circuits (i.e., nigrostriatal and raphe-striatal pathways).

3.3. Materials and Methods

3.3.1. Subjects

Adult female Sprague-Dawley rats (PN 65-74, 200-224g; N = 90; Harlan Laboratories, Indianapolis, IN) were double housed in a temperature-controlled room (22°C, 40 to 50% humidity) and subjected to a reverse light/dark cycle (14 hours light/10 hours dark; lights off at 10 a.m.). Each female was ovariectomized at the time of the lesion surgery and cannulation. An intrascapular estradiol silastic capsule [12.5% 17-β-estradiol benzoate, (E_2) 87.5% cholesterol; 10 mm in length; 0.058 in. I.D. × 0.077 in. O.D.; Dow Corning, Midland, MI] was also implanted during the same surgery. This concentration of E_2 in the capsules has been shown to mirror circulating levels of E_2 during proestrus (Mannino, South, Inturrisi, & Quiñones-Jenab, 2005). Systemic E_2 also enhances cocaine-mediated DA release (Becker & Rudick, 1999; Castner, Xiao, & Becker, 1993; Cummings et al., 2014) and cocaine-seeking behavior (Festa & Quiñones-Jenab, 2004; Russo, Festa, et al., 2003). Furthermore, studies examining cocaine-

mediated conditioned place preference generally use this type of hormone replacement to avoid differential cyclicity during conditioning (Sell et al., 2000). Animal husbandry, surgeries, and experiments were approved by the Institutional Animal Care and Use Committee at The University of Texas at Austin and were in accordance with the National Health Guidelines for the Use of Animals.

3.3.2. Surgery

3.3.2.1. Experiment 1

All lesions or sham lesions (n = 60) were performed using a Radionics radio-frequency lesion generator, with a TCZ thermo-coupled electrode (0.25 mm exposed tip). Surgeries were performed with the animals situated in a stereotaxic apparatus while still under general anesthesia directly after the ovariectomy. Bilateral lesions were aimed at the rostro-central mPOA [AP, -0.25 mm; ML, ±0.6 mm; DV, -8.3 mm; according to coordinates from Paxinos and Watson (2007)]. Once the electrode reached the mPOA, the temperature was raised to, and maintained at, +80°C (±3°C) for 20 seconds. This procedure was also done in a bilateral sham-treatment group, without introduction of radio frequencies. Animals were allowed a three-week recovery before beginning procedures for cocaine-induced cellular activity.

Ablations produced with radiofrequency-lesion generators are more reproducible in size and shape than are those produced with electrolytic or chemical techniques. For example, electrolytic lesions can vary up to a factor of four in terms of size and geometry, after fixed lesion current and time were applied (Sweet & Mark, 1953). The reason for this variability is that electrolytic lesions are influenced by electrolysis action, gas formation, anatomy of tissue planes, and nearby vascular interruption, which can vary

between animals. Lesions produced with radio frequencies do not present these problems, thus the radiofrequency technique was employed for our experiments.

After all tests were completed, lesion placements were verified histologically. Coronal brain sections including the mPOA were processed using methyl green, a Nissl stain that allows visualization of all cell nuclei. Any animal that did not have an mPOA lesion was removed from further analyses.

3.3.2.2. Experiment 2

All surgeries were done under isoflourane (Isosthesia™, Henry Schein Animal Health, Dublin, OH) anesthesia using sterile surgical protocols. The animals (n = 30) were placed into a stereotaxic apparatus, the skull was exposed via a mid-line incision and burr holes were drilled over the regions of interest along with three other burr holes that did not fully penetrate the skull and were used to anchor the surgical screws. Microinjections were done using a 1 mL gas-tight syringe on an infusion pump (Harvard Apparatus, South Natick, MA). The syringe was attached to polyethylene tubing (PE-20; Braintree Scientific, Inc., Braintree, MA), which was then attached to a 27-gauge thin-walled stainless-steel injection cannula. The injection cannula was lowered into the mPOA (coordinates: AP, +0.5mm; ML, -0.4; DV, -8.0mm from bregma) and 0.4μL of either N-methyl-D-aspartate (NMDA; n = 15; 25μg/μL; Sigma Aldrich, St. Louis, MO) dissolved in saline or saline alone (n = 15) was infused unilaterally. The infusions occurred over a 5-minute period (0.1 μL/min) and left in place for 5 minutes before withdrawal to allow for proper diffusion. After the injector was removed, a guide cannula (10mm; 23G stainless steel tubing; Small parts) was lowered into the ipsilateral cortex (coordinates: AP, +1.6 mm; ML, +1.1 mm; DV, -1.8 mm), 5mm above the most dorsal aspect of the NAc to allow for insertion of microdialysis probe during the experiment.

The guide cannula was secured onto the skull using a dental acrylic and skull screws that were placed into the previously drilled shallow burr holes. After completion of the surgery, the female was injected with gentamicin antibiotic (5mg/kg; company), ketoprofen (5mg/kg; company), and 1mL of 0.9% saline ringer (Hospira, Inc., Lake Forest, IL) to assist in recovery.

3.3.4. Experimental design

3.3.4.1. Experiment 1

Three weeks after the initial surgery, female rats were injected with either vehicle (0.9% saline; 1mL/kg; i.p.) or cocaine HCl (10 mg/kg; i.p.; Sigma Aldrich, St. Louis, MO), in a general use room adjacent to the vivarium. The rats were then returned to their home cages for 1 hr. This time period allowed for maximal expression of stimulus-mediated Fos. Once this time period elapsed, they were transferred to the necropsy room where they were euthanized, perfused with a fixative, and brains were removed.

3.3.4.2. Experiment 2

Microdialysis and behavioral tests were all done under red light illumination during the dark phase of the photocycle. The animals were allowed 3 weeks to recover from surgery before the experiment began. After probes were implanted, the rats were randomly placed into either the left or right chamber of a conditioned-place preference apparatus (San Diego Instruments Place Preference System, San Diego, CA), which allowed us to record gross locomotor activity in 15-minute bins via a laser beam-break system (full specifications described in Tobiansky et al., 2013). A black, opaque Plexiglas divider separated the left and right chambers in each apparatus. A 3-hr equilibration phase was allotted to allow DA and 5-HT to reach stable baseline levels (Ben-Shahar et al., 2013). Thereafter, samples were collected and gross locomotion was

recorded every 15 minutes. After 3 baseline samples, the rats received an i.p. injection of 0.9% saline ringer (1mL/kg) and three 15 min dialysate samples were collected. Finally, we injected a 10mg/kg dose cocaine HCl (i.p.; Sigma Aldrich, St. Louis, MO) and six 15 min dialysate samples were collected.

Probes were constructed according to methods used by Yamamoto and Pehek (1990). In brief, the probes were designed to have concentric flow and were constructed using a dialysis membrane (13,000 MW cutoff; Spectrum Labs, Rancho Dominguez, CA) with an outer diameter of 216 μm and an inner diameter of 200 μm. The active dialyzing length was 2 mm. The extraction fraction of each probe was measured *in vitro* before use to ensure maximal recovery of analytes. Only probes with recovery rates of > 10% were used. Filtered and degassed Dulbecco's PBS (in mM: 138 NaCl, 2.7 KCl, 0.5 $MgCl_2$, 1.5 KH_2PO4, and 1.2 $CaCl_2$; pH 7.4; Sigma Aldrich, St. Louis, MO) was perfused through the probe at a rate of 0.5 μL/min using a 1mL gas-tight syringe on a infusion pump (PHD 22, Harvard Apparatus, Holliston, MA). Dialysate samples were collected 15 in from the active zone 0.65 mL Eppendorf tubes. Samples were frozen in dry ice immediately after collection and stored in a -80°C freezer until analysis by high-performance liquid chromatography with electrochemical detection (HPLC-EC).

3.3.5. Tissue collection

The thoracic and abdominal cavities were exposed and the descending aorta was clamped. Rats were then perfused transcardially with 50 ml of 0.1M PBS followed by 250 ml of 4% paraformaldehyde in 0.1M PB. The brain was quickly removed, post-fixed for 1 hour in the same fixative. Thereafter, the brains were transferred to a solution containing 30% sucrose in 0.1M PB. Brains were stored and maintained at 4 ºC for at least 48-hrs until sectioning. For experiment 1, the brains were cut in coronal sections at

35 μm, whereas in experiment 2 they were cut at 100 μm through the NAc, and 50 μm through the mPOA. All sections were stored at – 20 °C in cryoprotectant solution containing 30% ethylene glycol, 30% sucrose, and 0.0002% sodium azide in 0.1M PB until immunoprocessing.

3.3.6. Histology and immunohistochemistry

3.3.6.1. Experiment 1

Free-floating sections were extensively rinsed in 0.1M PB between incubations. All incubations were performed at room temperature with gentle agitation. Sections were blocked with 1% H_2O_2 for 10 min and then soaked for 1-hr in incubation-block solution (0.1% BSA, 0.4% Triton-X, in 0.1M PB). Sections were then incubated overnight (16-hrs) with a polyclonal antibody recognizing Fos (rabbit anti-Fos, 1:5000; Santa Cruz Biotechnology, Santa Cruz, CA). After incubation with primary antibody, sections were exposed to biotin-conjugated goat anti-rabbit IgG (1:500 in incubation solution) for one hour. Secondary antibody was followed by incubation in avidin-biotin complex (Vector Laboratories, Burlingame, CA) for one hour; tissue was then transferred to a solution containing 0.02% 3,3'-diaminobenzidine (DAB), 0.1% H_20_2 in 0.1M PB, and 2% nickel sulfate. Extensive washing terminated this reaction. Fos-positive cells were counted in the regions of interest using NIH freeware Image J. Four sections for both the NAc core and shell [two rostral and two caudal; 0.65 mm_2 area for core (NAcC) and 0.50 mm^2 area for the shell (NAcS)] were arbitrarily chosen from each animal, and the immunoreactive cells were counted for each section.

3.3.4.2. Experiment 2

Free-floating coronal sections containing the mPOA from all animals were extensively rinsed in 0.1M PB between incubations. Incubations were done at room

temperature and used 0.1M PB as the diluent. After the primary wash, the tissue underwent a peroxidase block (1% H_20_2) for 10 min. A blocking solution that contained a mild detergent (2% normal goat serum, 2% bovine serum albumin and 0.04% Triton-x) was then introduced. Sections were then incubated overnight (18 hrs) in the blocking solution containing a monoclonal antibody for NeuN raised in mouse at a concentration of 1:16,000 (clone A60; EMD Millipore, Billerica, MA). Thereafter, sections were exposed to a biotinylated goat anti-mouse IgG (1:500; Vector Laboratories, Burlingame, CA) for 1 hr in blocking solution, followed by an incubation in an avidin-biotin complex (Vectastain elite ABC; Vector Laboratories, Burlingame, CA) for signal enhancement. Finally, the tissue was transferred to a solution containing 0.02% 3,3'-diaminobenzidine (DAB; Sigma Aldrich, St. Louis, MO), 0.1% H_20_2 and 0.04% $NiSO_4$. Extensive rinses in 0.1 M PB terminated the chromogen reaction. The immunostained tissue along with the untreated coronal slices containing the NAc were mounted simultaneously. All sections were counterstained with methyl green, dehydrated and coverslipped. Control sections for the mouse anti-NeuN antibody omitted the primary antibody, which resulted in no staining. Lesions and probe placement were verified under the microscope.

3.3.7. High performance liquid chromatography

The Antec Decade II chromatographic system (Antec Leyden, The Netherlands) was composed of a Valco Cheminert injector (Valco Instruments Co Inc., Houston, TX) with a 6-μL sample loop, a microelectrochemical detector in a microflow cell, and an Acclaim RSLC PolarAdvantage II analytical flow column (ThermoFisher Scientific, Waltham, MA), and was used for all neurochemical analyses. The analytical stationary phase was a reversed-phase capillary column (2.1 mm inner diameter, 100 mm long, packed with 2.2 μm C-18 particles). The system used an Antec VT-03 high sensitivity

flow cell with an ISAAC reference electrode for electrochemical detection. The working electrode was sustained at an applied potential of +0.50V relative to the reference electrode and the range was set at 200 pA. An Antec pump (Antec LC 110; Antec Leyden, The Netherlands) transported the mobile phase (12.5% HPLC-grade MeOH, 150 mg/L L-octanesulfonic acid, 8 mM NaCl, 0.1 mM ethylenediaminetetraacetic acid, 50 mM H_3PO_4; pH 5.5; filtered and vacuum degassed) through the system at a sustained flow rate of 0.225 mL/min. Data were collected with a computer running Clarity software (Data Apex, Prague, Czech Republic).

3.3.8. Statistical analyses

All data were analyzed using PASW statistical package (18th Ed). The numbers of Fos-immunoreactive cells were analyzed with an ANOVA or Kruskal-Wallis non-parametric test with Mann-Whitney U used to determine between group differences. Tukey post-hoc analyses were used to test between group differences if the interaction between the independent variables was significant. In the second experiment dependent measures were the gross locomotor scores and analyte concentrations. All dependent measures were analyzed with a two-factor, repeated measures (RM)-ANOVA) with group as the between-subject factor and time as the repeated within-subject factor. When appropriate, a Bonferroni *post hoc* test was performed using the Bonferroni correction as a protective measure in the multiple pairwise comparisons procedure. Statistical significance was set at $\alpha = 0.05$ for all analyses.

3.4. RESULTS

3.4.1. Experiment 1

Only animals with mPOA lesions were included in the analyses (n = 54) To determine whether cocaine has an influence on activity in the mPOA, we examined

cocaine-mediated Fos-ir in the rostral mPOA. Analyses were constrained to the rostral mPOA because in a previous study (see Chapter 2) we found that cocaine increases Fos-ir in preoptotegmental perikarya in this subregion of the mPOA. The data revealed a main effect of acute cocaine ($t_{(1,23)} = 2.304$, $p < .05$; Figure 3.1), wherein cocaine increase Fos-ir in the rostral mPOA.

To help establish a functional connection between the mPOA and cocaine-induced mesolimbic activity, we compared Fos- positive immunoreactivity in the NAc of rats with or without radiofrequency lesions of their mPOA, following intraperitoneal cocaine (10 mg/kg) or vehicle injections. Consistent with previous findings (Graybiel, Moratalla, & Robertson, 1990; Zahm et al., 2010), cocaine administration in intact rats activated the NAc, as evidenced by an increased number of Fos-positive cells versus vehicle controls. However, rats with lesions of their mPOA had a significantly greater response to cocaine versus rats with sham lesions. Specifically, removal of the mPOA augmented cocaine-induced activity in the NAc (see Figure 3.2); this effect was observed in both the NAc core (NAcC; $F_{(1,50)} = 8.978$, $p < .01$) and shell regions (NAcS; $F_{(1,50)} = 9.105$, $p < .01$).

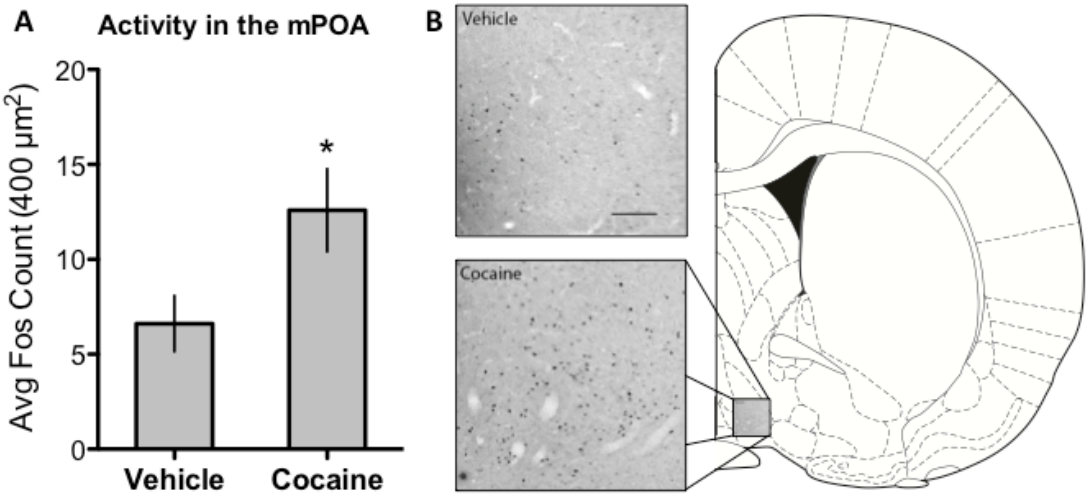

Figure 3.1. Acute cocaine enhances Fos expression in the rostral mPOA. (A) A graph demonstrating that Fos-ir is seen in higher levels in the rostral mPOA in animals that received acute cocaine versus animals treated with vehicle. (B) Representative photomicrographs of Fos-ir of vehicle and cocaine treatments in the rostral mPOA. Scale bar, 50 µm; values are expressed as mean ± SEM. *$p < .05$. Coronal plate was adapted from The Rat Brain in Stereotaxic Coordinates (6th ed.).

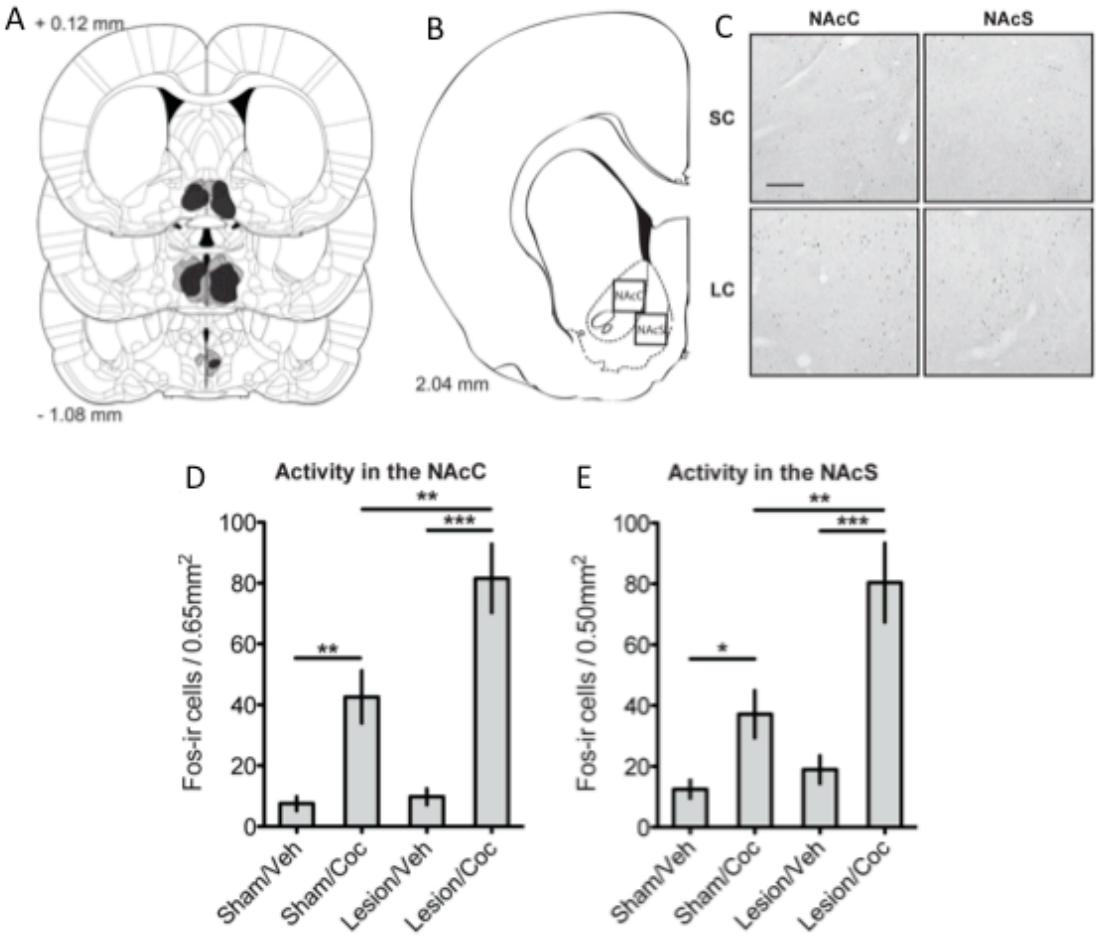

Figure 3.2. Lesions of the mPOA increased cocaine-induced activity in the NAc. (a) Representative perimeters of smallest (black lines) and largest (gray lines) lesion for rats with radiofrequency lesions of their mPOA, drawn from Paxinos and Watson (2007). (b) Representative photomicrographs of Fos-ir in the NAcs of animals receiving cocaine or vehicle injections and lesion or sham-lesion of their mPOA. (c) Graph demonstrating the number of Fos-positive cells in the NAcc and NAcs across treatment conditions. Scale bar, 50 μm; values are expressed as mean ± SEM. *$p < .05$, **$p < .01$, ***$p < .001$. Coronal plates were adapted from The Rat Brain in Stereotaxic Coordinates (6th ed.).

3.4.2. Experiment 2

3.4.2.1. Locomotor behavior

All animals included in the data analyses were checked for lesion placement (Figure 3.3.A). Histological analysis of lesions revealed that 9 of 15 (60%) were

successful. Of the 9 lesions most were localized in the central mPOA. Furthermore, uterine horn widths did not differ ($t_{(19)} = 0.24$, $p = 0.81$) between sham lesion (5.46 ± 0.15; n = 13) and lesion (5.56 ± 0.47; n = 8) groups, suggesting similar levels of circulating estradiol. Lesions of the mPOA did not significantly influence baseline, saline-induced, or cocaine-induced locomotion compared to sham lesioned animals. In particular, raw locomotor counts ($F_{(1,220)} = 2.62$, $p = 0.12$) and locomotor counts as a percent of baseline ($F_{(1,220)} = 2.62$, $p = 0.12$) did not demonstrate significant between-group differences. Not surprisingly, there were significant main effects when examining the within-subject factor, time, in both raw locomotor counts ($F_{(11,220)} = 29.68$, $p < 0.0001$) and locomotor counts as a percent of baseline ($F_{(11,220)} = 29.68$, $p < 0.0001$). No significant interactions were observed.

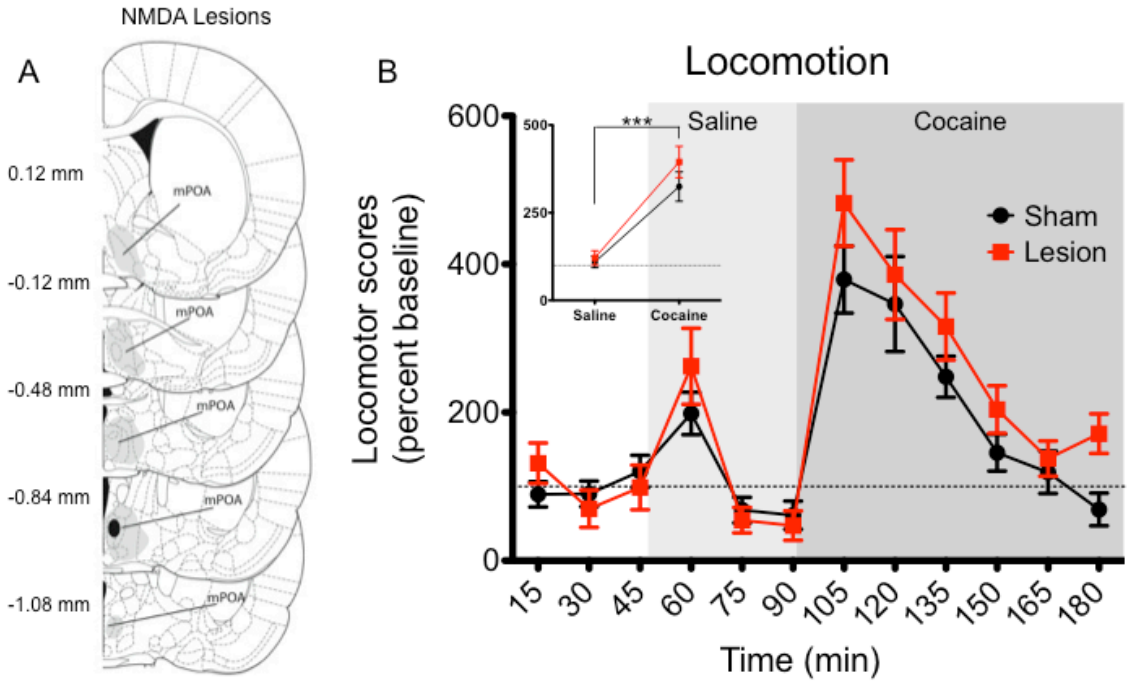

Figure 3.3. mPOA lesions do not influence cocaine-mediated locomotion. (A) Coronal hemisection plates demonstrating the largest acceptable lesion size (gray), and the smallest acceptable lesion size (black) in the mPOA. Plates were adapted from The Rat Brain in Stereotaxic Coordinates (6th ed.) by G. Paxinos & C. Watson, 2007, New York, NY: Academic Press. (B) Saline and cocaine injections have similar effects on locomotion scores represented as a change from baseline between sham lesioned (n = 13) and lesioned groups (n = 9). Inset demonstrates a main effect of cocaine-injection on locomotion for both groups. Values represent the average of the three collapsed time bins directly after each injection (i.e., saline = 60-90 min; cocaine = 105-135 min). All values are represented as the mean ± SEM. ***$p < 0.001$

3.4.2.1. Neurochemical responses in the NAc

Overall, there was no significant difference ($t_{(15)} = 0.40$, $p = 0.69$) of probe recovery between probes used in the sham lesion (17.18 ± 4.99) and lesion (20.09 ± 4.83) groups. All 13 sham animals and 9 lesion animals used for locomotor and dialysate analyses had probe placements in the NAc, with the majority (50.0%) in the shell region

of the NAc, 40.9% in the shell/core border, and 9.1% in the core. There were no between-group differences in subregional placement of the probe ($t_{(20)} = 0.75$; $p = 0.46$)

Analyses of percent change of DA from baseline revealed a significant time × lesion group interaction ($F_{(11, 220)} = 2.537$; $p < 0.05$). Bonferroni post hoc tests showed significant differences between groups at the two time bins immediately following cocaine injection (105 min, $p < 0.05$; and 120 min, $p < 0.01$). Finally, a RM-ANOVA examining collapsed saline and cocaine-induced levels of DA as a percent baseline revealed a significant main effect of cocaine treatment ($F_{(1,20)} = 25.72$; $p < 0.0001$). There was one animal that had a notably higher percent baseline DA level in the lesion group at 75 min, 30 min after the initial saline injection, which increased the variance at that time point. At first glance, this appears to be a lesion-specific response to stress, but the lack of statistical separation between groups suggests otherwise. As for 5-HT, a two-way RM-ANOVA exposed a main effect of time ($F_{(11,156)} = 5.07$; $p < 0.0001$), suggesting a cocaine effect, but no significant main effect of lesion ($F_{(1,165)} = 0.23$; $p = 0.64$). Similar to the DA analysis, a two-way RM-ANOVA for drug treatment demonstrated a significant main effect of cocaine ($F_{(1,15)} = 14.63$; $p < 0.01$). See Figure 3.4 for a summary of these findings.

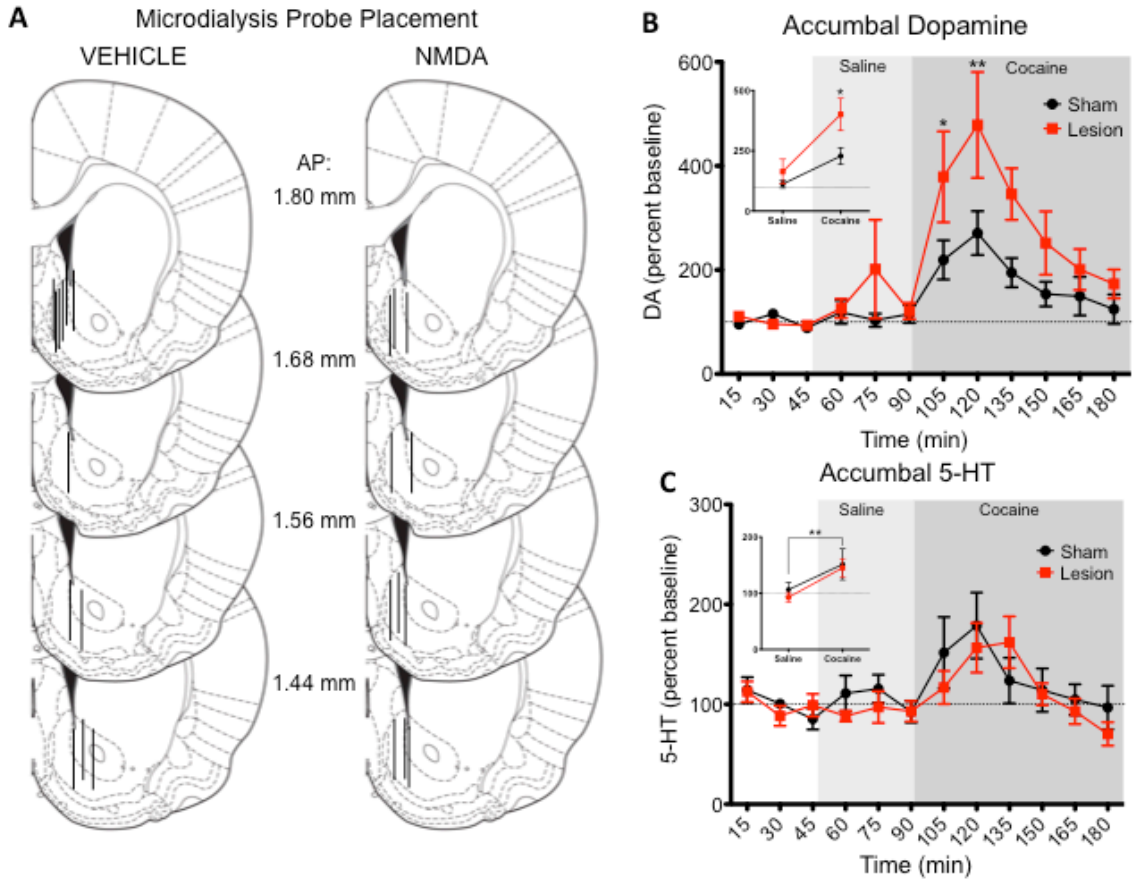

Figure 3.4. mPOA lesions enhance cocaine-mediated DA release. A, DA release changes in percent from baseline between sham lesioned (n = 13) and lesioned (n = 9) female rats. Group differences are seen time points 105 (sham: 219 ± 37%; lesion: 379 ± 87%) and 120 (sham: 271 ± 42%; lesion: 478 ± 102%), which were the two dialysate samples collected directly after the cocaine injection. Inset shows an interaction as a function of average percent baseline DA levels between the three saline and the first three cocaine time points collapsed between groups. B, Saline- and cocaine-mediated 5-HT release represented as a change in percent from baseline between sham (n = 10) and lesion (n = 7). The inset is organized the same way as the inset in 2A and shows a main effect of cocaine treatment but no effect of lesion. All values are represented as the mean ± SEM. *p < 0.05, **p < 0.01

3.5. DISCUSSION

The goal of this study was to characterize the role of the mPOA in modulating cocaine-induced activity and DA release in the mesolimbic system in female rats. Literature suggests that the mPOA intercedes a variety of DA-dependent naturally reinforcing behaviors (see Hull, 2011 for review; Moses, Loucks, Watson, Matuszewich, & Hull, 1995; Pfaus & Scepkowski, 2005) and may play a role in the reinforcing aspects of cocaine (Mattson & Morrell, 2005; Pereira & Morrell, 2011; Tobiansky et al., 2013). Yet, most if not all reviews and meta-analyses on the mesolimbic DA system and psychostimulants do not include the mPOA as a brain region of import (see Björklund & Dunnett, 2007; Koob & Volkow, 2010; Nestler, 2005 for examples). Results shown above accentuate the need to include it as such.

This is the first report, to the best of our knowledge, demonstrating that the mPOA directly regulates cocaine-induced activity and DA release in the NAc in rats. In particular, lesioned animals displayed an enhancement of cocaine-mediated Fos-ir in both the shell and core of the NAc. Moreover, the female rats exhibited similar changes in percent baseline of DA and 5-HT in response to an acute stressor (i.e., saline injection) compared to their sham lesion counterparts. The mPOA has been implicated in stress responsivity (Herman & Cullinan, 1997) and the NAc has been associated with stress and aversion responses (Carlezon & Thomas, 2009; Ilango, Kesner, Broker, Wang, & Ikemoto, 2014; Liu, Shin, & Ikemoto, 2008). Yet, the accumbal DA response to injection stress is rapid (< 5 min), and is too transient for detection by *in vivo* microdialysis (Ventura, Cabib, & Puglisi-Allegra, 2001). Importantly, there were similar percent baseline levels of stress-induced and cocaine-induced locomotion and 5-HT release. The implication being that this enhanced DA response is specific to the reinforcing aspects of

cocaine and not its influence on motoric (Leavitt, 1969) or serotonergic systems (Reith, Li, & Yan, 1997; Teneud, Baptista, Murzi, Hoebel, & Hernandez, 1996).

Due to the nature of lesion studies, we can only conjecture on how mPOA lesions amplify DA release in the mesolimbic system. The mPOA is composed of a heterogeneous population of neurons. Previous studies have shown that the primary amino acid produced by preoptic perikarya is γ-aminobutyric acid (GABA; Herbison et al., 1992; Unda, Brann, & Mahesh, 1995). While there are glutamate-producing cells, GAD_{67}-containing (an enzyme responsible for GABA production) cells are three times as prevalent (Tsuneoka et al, 2012). Furthermore, maternal behaviors increase putative activity of GABA-producing cells in the mPOA of virgin (Tsuneoka et al., 2012) and lactating female rats (Lonstein & De Vries, 2000). Efferent targets of these fos-positive cells were unknown. A previous study in our lab determined that the majority (67.6 ± 6.5%) of rostral and central preoptotegmental efferents are GABAergic, there are GABAergic terminals originating from the mPOA in the VTA (Tobiansky et al., 2013) and preoptotegmental efferents appose DAergic tegmentostriatal neurons (see Chapter 2). Moreover, cocaine enhances putative activity in the rostral mPOA (Tobiansky et al., 2013; Mattson & Morrell, 2005). In a separate study we found that cocaine increases the number of Fos-positive cells in rostral mPOA-to-VTA perikarya (unpublished results). There has yet to be a study to determine if these GABAergic efferents are the cells that are being activated by cocaine or stimuli associated with maternal behavior. Even if cocaine and natural rewards activate these GABAergic efferents, the net effect would be difficult to predict due to the phenotypic heterogeneity of VTA cells (Ikemoto, 2007). Efferent fibers extended throughout the VTA including the GABAergic tail (VTT) and the DA-rich parabrachial pigmented and paranigral nuclei (see Chapter 2). Notwithstanding, the data presented here demonstrates a pivotal role for the mPOA in

regulating net DA output in the NAc, meaning that the elimination of the mPOA enhances cocaine-mediate activity in VTA DA neurons regardless of subregional VTA connections.

3.6. SUMMARY AND CONCLUSIONS

The findings presented here along with previous reports from our lab hold implications for understanding cocaine abuse in women. Cocaine use, addiction, and treatment, have been on the rise in women (Greenfield et al., 2003; SAMHSA, 2011). Women use cocaine at similar rates when their ability to access the drug is the same as men (Van Etten et al., 1999). They also begin using at a younger age (Griffin et al., 1989) and have shorter abstinence periods (Kosten et al., 1996). As it is impossible to do in humans, animal research has been indispensible in showing that female rats differ in rates of cocaine acquisition. Females self-administer cocaine and exhibit cocaine-mediated conditioned place preference faster and at lower doses than males (Lynch & Carroll, 1999; Lynch et al., 2001; Lynch, 2008; Russo, Jenab, et al., 2003). Given that the sexually dimorphic mPOA plays a role in sex-specific responses to naturally reinforcing stimuli (Edwards & Einhorn, 1986; Graham & Pfaus, 2010, 2012; Miller & Lonstein, 2005; Will et al., 2014; Yahr & Greene, 1992), this research may help us understand how the mPOA influences sex-specific responses to cocaine-mediated reinforcement and inform clinical studies on cocaine abuse.

Chapter 4: Effects of mPOA lesions on conditioned approach behavior[3]

4.1. ABSTRACT

Drugs of abuse exert their effects by exploiting natural neurobiological reward mechanisms, especially the mesolimbic dopamine (DA) system. However, the mesolimbic system does not operate in isolation, and input from other reward-relevant structures may play a role in cocaine's rewarding effects. The medial preoptic area (mPOA) of the hypothalamus is involved in the regulation of two essential and naturally rewarding behaviors: sexual and maternal behaviors. It also makes strong neuroanatomical connections with areas of the mesolimbic system, particularly the ventral tegmental area (VTA). As such, the mPOA is a logical candidate for a neuroanatomical locus modulating activity in the mesolimbic system and emergent behavioral expressions of drug reward, yet the role of this structure is largely unexplored. Here, using a female rat model, we show that lesions of the mPOA augment cocaine-conditioned place preference. This suggests a novel key role for the mPOA in the inhibition of the mesolimbic DA circuit. These results reveal the mPOA as a critical modulating structure in regulating the incentive salience of cocaine.

4.2. INTRODUCTION

Appropriate behavioral responses to natural reward are necessary for survival and reproductive success. To this end, neurobiological reward mechanisms have evolved to reinforce behaviors such as sexual interactions and parental care. However, drugs of abuse, such as cocaine, subvert this natural reward/reinforcement system. In particular, the mesolimbic system is recognized as playing a critical role in reward, and cocaine

[3] Portions of text in this section are excerpted from Tobiansky DJ, Roma PG, Hattori T, Will RG, Nutsch VL, Dominguez JM, Behavioral Neuroscience 127(2), 293-302. PGR assisted in establishing the conditioned place preference paradigm in our lab. TH, RGW, VLN and JMD were involved in edits and assisted in surgery.

exerts its psychostimulant effects by producing activity in this pathway (Kalivas & Duffy, 1990; Koob, Sanna, & Bloom, 1998; Nestler, 2005; Wise, 2002). However, this system does not operate in isolation, and input from other reward-relevant structures may play a critical role in cocaine-induced activity.

An area that is vital for natural reward but has received little attention as a potential modulator of cocaine-induced activity is the medial preoptic area (mPOA) of the hypothalamus. The hypothalamus is well known for its role in motivated behaviors and reinforcement, and the mPOA in particular is critically involved in the regulation of two essential and naturally rewarding functions — sexual (Hull & Dominguez, 2007) and maternal (Lonstein, 2002) behaviors. It also strongly interacts with areas of the mesolimbic system, particularly the ventral tegmental area (VTA; Simerly & Swanson, 1988; Zahm et al., 2011). As such, the mPOA is a logical candidate for a neuroanatomical locus modulating cocaine-induced activity, yet surprisingly little is known about the role of this structure in the context of drug abuse. This is a critical gap in knowledge because the mPOA is also a central integrative region for gonadal hormone stimulation, and, as such, studies elucidating on its role in drug response will shed light on mechanisms through which hormones might impact drug activity.

The purpose of the present study was to determine whether the mPOA modulates cocaine-seeking behavior. To this end, female rats received either radiofrequency lesions of the mPOA or sham lesions and were exposed to a Pavlovian conditioning paradigm, conditioned place preference, to determine whether the mPOA influences the salience of cocaine. We hypothesized, that, because lesions of the mPOA generally result in deficits of behaviors in response to naturally rewarding stimuli, these radiofrequency lesions would attenuate cocaine salience. Results obtained in these experiments will help resolve whether the mPOA modulates neural and behavioral responses to cocaine.

4.3. Materials and Methods

4.3.1. Subjects

Adult female Sprague–Dawley rats (Harlan Laboratories, Indianapolis, IN) were singly housed in a climate-controlled room (22°C; 40 to 50% humidity) on a reverse light–dark cycle (14:10; lights off at 10 a.m.), with food and water freely available. All rats were ovariectomized and implanted with an intrascapular estrogen silastic capsule (5% 17β-estradiol benzoate, 95% cholesterol; 12 mm in length; 1.98 mm I.D. × 3.18 mm O.D.; Dow Corning, Midland, MI). Procedures were in accordance with the National Institutes of Health Guidelines for the Use of Animals in Research and were approved by the University of Texas at Austin Institutional Animal Care and Use Committee

4.3.2. Lesions of the mPOA

All lesions or sham lesions (n = 44) were performed using a Radionics radio-frequency lesion generator, with a TCZ thermo- coupled electrode (0.25 mm exposed tip). Surgeries were performed with the rats situated in a stereotaxic apparatus while still under general anesthesia, immediately after the ovariectomy. Bilateral lesions were aimed at the rostrocentral mPOA (AP, -0.25 mm; ML, ±0.6 mm; DV, -8.3 mm; according to coordinates from Paxinos and Watson, 2007). Once the electrode reached the mPOA, the temperature was raised to, and maintained at, +80°C (±3 °C) for 20 s. This procedure was repeated for the bilateral sham-treatment group, without introduction of radiofrequency. Rats were allowed a 3-week recovery period before beginning procedures for cocaine-induced conditioned place preference.

After all tests were completed, lesion placements were verified histologically. Coronal brain sections including the mPOA were processed using methyl green, a Nissl

stain that allows visualization of all cell nuclei. Any rat that did not have an mPOA lesion was removed from further analyses.

4.3.3. Conditioned place preference

We utilized a fully biased place-conditioning paradigm using automated two-chambered conditioned place preference (CPP) apparatuses (San Diego Instruments Place Preference System, San Diego, CA). The inner dimensions of each conditioning chamber were 35 cm wide × 21 cm deep × 34.5 cm high. The left chamber featured a smooth black plastic floor, whereas the right chamber featured a haircell-textured black plastic floor. Each individual chamber featured an overhead array of experimenter- operated switchable and dimmable white and red LED lights. The red LED light was at maximum intensity on the side with the smooth floor. Each apparatus contained a 16 × 4 photobeam array for recording time spent in each chamber (in seconds), number of chamber transitions, exploratory approaches to adjacent chambers, gross locomotor activity (consecutive breaks of adjacent beams), and fine motor activity (repeated breaks of the same beam).

All rats were allotted 20 min free access to the entire apparatus as a baseline-preconditioning test (PRE test session). For conditioning, drug-paired chamber assignments were based on the rats' initial non-preferred side during the PRE. For the conditioning cycles, all rats received an intraperitoneal (i.p.) injection of saline (1 mL/kg) and were secluded to their initially preferred chamber for 30 min. Immediately thereafter, they received either a 10 mg/kg i.p. injection of cocaine, or equivolume saline for control rats, followed by confinement to their respective drug-paired chambers for 30 min. We repeated this pattern for two conditioning cycles, and then provided each rat 20 min of access to the entire apparatus to assess preferences for the drug-paired chamber (POST

test session). The four groups consisted of those having sham lesions and vehicle injection (SV; n = 12), sham lesions and cocaine injection (SC; n = 13), mPOA lesions and vehicle injection (LV; n = 10), or mPOA lesions and cocaine injection (LC; n = 9).

4.3.4. Tissue collection

One to three days after the CPP POST rats were euthanized with a lethal dose of Euthasol (0.3 mL/animal, Virbac Animal Health, Inc., Fort Worth, TX). The thoracic and abdominal cavities were exposed and the descending aorta was clamped. Rats were then perfused with 50 ml of 0.1M PBS, followed by 250 ml of 4% paraformaldehyde in 0.1M PB via a transcardial puncture. The brain was quickly removed, postfixed for 1 hr in the same fixative. Thereafter, the brains were transferred to a solution containing 30% sucrose in 0.1M PB. Brains were stored and maintained at 4 °C for at least 48 hr until sectioning. Coronal sections, cut at 35 μm, were stored at –20 °C in cryoprotectant solution containing 30% ethylene glycol, 30% sucrose, and 0.0002% sodium azide in 0.1M PB.

4.3.5. Statistical analyses

All data were analyzed using PASW statistical package (18th ed.). The conditioned place preference POST was analyzed via ANCOVA with total ambulation as the covariate. Tukey post hoc analyses were used to test between group differences if the interaction between the independent variables were significant. Statistical significance was set at $\alpha = .05$ for all analyses

4.4. RESULTS

To address the issue of whether the increased neural activity previously described translates into behavioral expression of reward, we utilized cocaine-induced CPP. Results showed that lesions of the mPOA indeed increased the effects of cocaine on reward, as

evidenced by a greater cocaine-induced preference in rats with mPOA lesions versus both their vehicle-treated lesioned counterparts and their cocaine-treated sham-lesioned controls ($F_{(1,50)} = 9.105$, $p < .01$; see Figure 4.1. for a more detailed description). In sum, this experiment reveals a modulatory role for neural mechanisms in the mPOA on cocaine responses upstream in the mesolimbic system and subsequent behavioral manifestation of incentive salience.

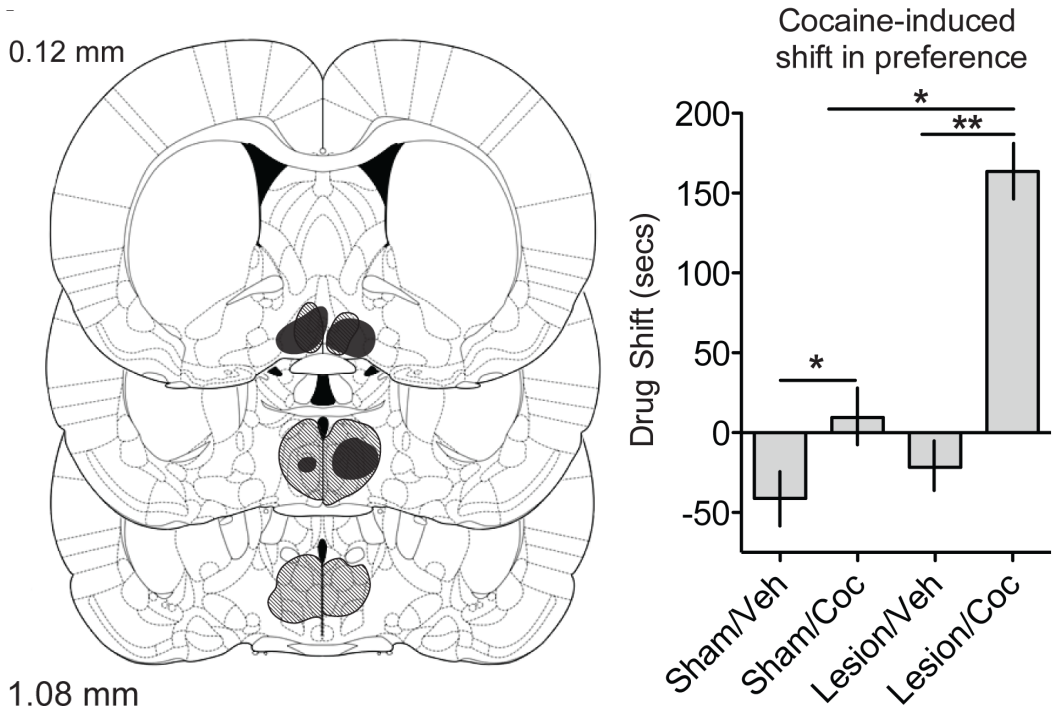

Figure 4.1. Lesions of the mPOA increased cocaine-induced conditioned place preference. (A) Representative perimeters of smallest (black lines) and largest (gray lines) lesion for animals with radiofrequency lesions of their mPOA, drawn from Paxinos and Watson (2007). (B) Graph demonstrating the shift in chamber preference across treatment conditions, cocaine treated animals displayed a cocaine-induced shift in time spent in an initially non-preferred chamber, whereas animals receiving lesions of the mPOA displayed an even greater shift. Values are expressed as mean ± SEM. *$p < .05$, **$p < .01$. Coronal plates were adapted from The Rat Brain in Stereotaxic Coordinates (6th ed.), Fig 3a from pages 75, 80, and 84, by G. Paxinos & C. Watson, 2007,

4.5. DISCUSSION

The findings presented here reveal, that the mPOA modulates cocaine-induced behavioral activity. We discovered that lesions of the mPOA enhanced behavioral expression of cocaine reward, presumably due to the removal of inhibitory mPOA efferents to the VTA. Taken together, the present study reveals a robust modulatory role of the mPOA in cocaine-induced behavior.

It is well established that DA in the NAc, which arises from neurons residing in the VTA, plays a vital role in the regulation of drug and natural reward, including reproductive and maternal behaviors. Studies demonstrating that mating activates cells in the NAc and increases DA levels support this conclusion (Hull & Dominguez, 2007; Meisel et al., 1993). For example, NAc DA and Fos activity increase in anticipation of sexual activity and further increase during mating in males and females (Damsma, Pfaus, Wenkstern, Phillips, & Fibiger, 1992; Jenkins & Becker, 2003; Pfaus & Phillips, 1991). As with sex, DA is also integral to the expression of maternal behaviors. Moreover, studies show that presenting pups to a lactating dam increases levels of DA (Hansen et al., 1993) and Fos activity (Fleming, Suh, Korsmit, & Rusak, 1994) in the NAc, whereas removal of DA fibers or pharmacological blockage of DA receptors in the NAc impairs maternal behaviors (Hansen, 1994; Keer & Stern, 1999). These and other studies establish mesolimbic activity as a critical component in the regulation of these two naturally rewarding behaviors.

By artificially stimulating these same neuronal mechanisms, drugs of abuse, such as cocaine, appropriate the natural reward system. Supporting this conclusion is evidence that rats will self-administer amphetamine (Hoebel, Hern, Aulisi, Stanley, & Lenard, 1983; Phillips, Robbins, & Everitt, 1994), DA reuptake inhibitors (Carlezon et al., 1995), and DA receptor agonists directly into the NAc (Ikemoto & Glazier, 1997). A similarly rewarding effect is observed using CPP: When given a choice between an environment previously paired with microinjections of DA agonists into the NAc or an environment paired with vehicle injections, animals prefer the drug-paired environment (Carr & White, 1983, 1986; White, Packard, & Hiroi, 1991). Given that cocaine increases DA in the NAc, and lesions of DA fibers in the NAc severely inhibit cocaine self-administration and CPP (Lyness, Friedle, & Moore, 1979; Pettit et al., 1984), it has been concluded that

activity in the mesolimbic system serves as a critical mechanism responsible for the psychostimulant effects of the drug, much in the same way as it does for natural reward.

Activity of VTA DA neurons is governed by inhibitory input acting on $GABA_A$ and $GABA_B$ receptors, excitatory input acting on N-methyl-D-aspartate (NMDA) and non-NMDA glutamate receptors, and dopamine (DA) input acting on primarily D_2-like receptors (Johnson & North, 1992). Of neurons residing in the VTA, approximately 65% are DArgic (Margolis et al., 2006; Nair-Roberts et al., 2008), which give rise to DA in the NAc (Fallon & Moore, 1978; Phillipson & Griffiths, 1985; Swanson, 1982). Consequently, modulation of these cells leads to dynamic changes in DA in the NAc and ensuing reward responses following a natural or drug stimulus. The prominence of GABA's influence on DA neurons in the VTA for NAc DA and stimulus reinforcement is now well established (Jhou, Fields, Baxter, Saper, & Holland, 2009; Johnson & North, 1992; Laviolette & van der Kooy, 2001; Shank, Seitz, Bubar, Stutz, & Cunningham, 2007; Stobbs et al., 2004), as several studies illustrate the importance of these mechanisms. Studies using intracranial self-administration show that rats will self-administer a $GABA_A$ receptor ($GABA_AR$) antagonist (picrotoxin) into the anterior VTA, but not the substantia nigra or other areas near the VTA (Ikemoto, Murphy, & McBride, 1997). Ikemoto and colleagues (1997) also showed increased DA in the NAc of rats that received picrotoxin into the anterior VTA (Ikemoto, Kohl, & McBride, 1997). Coinfusion of muscimol, a $GABA_AR$ agonist, reduced both the intracranial self-administration of picrotoxin and the increase release of DA in the NAc (Ikemoto et al., 1997; Ikemoto et al., 1997). A more recent study performed in mice showed that optogenetic activation of VTA GABA neurons suppresses DA neurons in the VTA and the release of DA in the NAc, while also disrupting sucrose consumption (van Zessen, Phillips, Budygin, &

Stuber, 2012). These and other findings establish that disinhibition of VTA DA neurons increases extracellular DA in the NAc and is reinforcing.

A wealth of pharmacological, neurochemical, and endocrinological evidence demonstrates a very important role for gonadal hormones in modulating rewarding stimuli, including cocaine (Carroll et al., 2004; Evans et al., 2002; Larson et al., 2007; Lynch et al., 2006, 2002; Russo, Festa, et al., 2003). However, the neuroanatomical locus of gonadal hormone influences on cocaine effects remains uncertain. Although our experiments did not explicitly examine hormonal action, it is still possible to speculate on the neuroendocrine implications of our findings. The highest concentration of estrogen (Shughrue, 1998; Simerly et al., 1990), progesterone (Hagihara, Hirata, Osada, Hirai, & Kato, 1992; Quadros, Pfau, & Wagner, 2007), and androgen receptors (Simerly et al., 1990) in the CNS are found primarily in the mPOA, which is a known neuroanatomical locus through which gonadal hormones act to modulate natural reward (Hull et al., 1999). Our results now show that the mPOA also modulates cocaine reward. Given its abundant receptivity to gonadal hormones, ongoing and future experiments will examine whether the mPOA is a window via which gonadal hormones impact cocaine reward responses, in much the same way that it influences other naturally rewarding processes.

Finally, we should like to comment on cocaine and sex-sensitive differences. Cocaine affects females differently than males (hyBecker et al., 2001; Lynch et al., 2002). In light of our results, this is significant because the mPOA contains the first discovered sexually dimorphic nucleus (SDN); the SDN of the preoptic area (SDN-POA) is structurally 2 times larger in male rats than in female rats (Gorski et al., 1978, 1980). Sexual dimorphism persists in the mPOA even to the level of the synapse, as female rats have more synapses on dendritic spines and fewer on shafts than do males (Raisman & Field, 1971). A homologous SDN exists in the human hypothalamus (Swaab, Chung,

Kruijver, Hofman, & Ishunina, 2001), which is also larger in men than in women. This dimorphism is important to note because females have an increased response to cocaine after estrogen replacement, whereas males do not have this same response. Here, we showed a modulatory role for the mPOA in cocaine-induced activity; therefore, it is reasonable to expect that in this capacity, the mPOA may also influence gender-sensitive differences in cocaine activity. Ongoing and future experiments will examine whether the mPOA modulates these gender differences.

4.6. SUMMARY AND CONCLUSIONS

In conclusion, a functional link between the mPOA and mesolimbic system was established with lesions of the mPOA, which enhanced the behavioral expression of cocaine reward, presumably due to the removal of the inhibitory mPOA efferents to the VTA. The present data suggest a modulatory role of the mPOA in cocaine-induced neural and behavioral activity.

Chapter 5: General Discussion

There are significant and consistent sex differences in cocaine seeking and consumption behaviors in humans (Evans & Foltin, 2006; Fattore, Melis, Fadda, & Fratta, 2014; Sofuoglu et al., 1999), non-human primates (Evans & Foltin, 2010) and rodents (Hu & Becker, 2003; Lynch et al., 2000; Lynch & Carroll, 2000). In particular, circulating E_2 enhances, while circulating P_4 attenuates, all phases of cocaine abuse (Bobzean, Dennis, et al., 2014; Caine et al., 2004; Carroll & Anker, 2010; Lynch, 2008). These differences are mediated, in part, by interactions between circulating sex steroid hormones and activity in the mesolimbic dopamine (DA) system (Becker & Cha, 1989; Becker, 1999; Cummings et al., 2014). Yet, there is a paucity of traditional nuclear receptors for E_2 and P_4 in the VTA and the ventral striatum, suggesting that these ovarian hormones may act on the mesolimbic DA system through a secondary pathway. While there are membrane-associated receptors in the VTA and NAc that may assist in mediating the effects of these sex steroid hormones (Becker, 1999), other neural nodes that are especially sensitive to sex steroid hormones and interact with the mesolimbic DA system may also be responsible for hormonal mediation of cocaine-associated reward. The main goal of this dissertation was to determine whether the mPOA plays a role as a secondary node in mediating the rewarding properties of cocaine, and possibly other drugs of addiction.

The existence of connections between the mPOA and the mesolimbic system had already been well established by several labs prior to the initiation of the experiments presented in this dissertation (Fahrbach et al., 1986; Ikemoto & Bonci, 2014; Simerly & Swanson, 1986, 1988). Our findings are consistent with their findings and, more importantly, we further defined the neuroanatomy and phenotypic profile of this circuit.

In particular, this is the first report to demonstrate that there is a rostrocaudal gradient of perikarya within the mPOA that then project to the VTA, whereby the rostral and central mPOA project heavily to the VTA and the caudal mPOA lacks connectivity to the VTA. Drawing on studies from quail and rodents, Balthazart and Ball (2007) proposed that the rostral mPOA is important for appetitive sexual behaviors, the central mPOA is important for both appetitive and consummatory sexual behavior, and the caudal mPOA is involved in regulating consummatory sexual behavior. The rostrocaudal gradient of preoptotegmental efferents reported in this dissertation suggests that the rostral and central mPOA may mediate the rewarding aspects of appetitive and consummatory behaviors through its connectivity to the VTA. Concurrently, these efferents, particularly within the central mPOA, project equally to DA rich regions and GABAergic tail of the VTA. Regardless of the neurotransmitter content of preoptotegmental efferents, this uniform projection to DAergic and GABAergic regions of the VTA makes it difficult to determine how these inputs may affect tegmentostriatal DA release. With the advent of optogenetics, future studies may be able to look at how afferents from the mPOA in the VTA influence DA release in the NAc and subsequent conditioned approach behavior.

Nevertheless, we found that preoptotegmental efferents are mainly GABAergic, suggesting that it plays an inhibitory role in mediating activity in the VTA. This is in line with previous studies demonstrating that the majority of perikarya in the mPOA are GABA-producing cells (Herbison et al., 1992, 1995; Tsuneoka et al., 2013). In addition, the majority of these GABAergic preoptotegmental efferents are responsive to DA as they express D_2-like receptors. Sensitivity to DA signaling in these efferents is relevant for several reasons. First, the mPOA receives DAergic input from a variety of diencephalic and mesencephalic brain regions, including, but not limited to, the incertohypothalamic areas, the substantia nigra, and the VTA (Miller & Lonstein, 2009;

Wagner, Eaton, Moore, & Lookingland, 1995). Secondly, DA agonists and antagonists microinfused into the mPOA affect the expression of maternal (Miller & Lonstein, 2005) and female sexual behaviors (Dean-Graham & Pfaus, 2010, 2012). Together, this suggests that DA originating from several neural nodes acts in the mPOA, possibly through efferents to the VTA, to mediate naturally rewarding behaviors. Finally, cocaine is a DAT inhibitor that increases DA levels in the synaptic cleft (Kuhar et al., 1991; Ritz et al., 1987; Wilcox et al., 1999). Given that preoptotegmental efferents are DA-responsive via D_2Rs and mainly GABAergic, cocaine-mediated increases in DA would likely inhibit mPOA activity, as activation of D_2R leads to hyperpolarization of the cell membrane; (Missale, Nash, Robinson, Jaber, & Caron, 1998; Stoof & Kebabian, 1981), which would ultimately disinhibit the VTA. Because preoptotegmental efferents project equally to GABAergic interneurons and DAergic cells in the VTA, these neuroanatomical results allow for different mechanisms of mesolimbic modulation. Notwithstanding, results presented here demonstrate that almost half of DA-producing cells in the VTA that project to the shell of the NAc are apposed by fibers originating from mPOA cell bodies; suggesting that cocaine-mediated hyperpolarization of GABAergic cells in the mPOA may enhance DA release in the NAc.

These mPOA cells are not only GABAergic and DA-sensitive but they are also responsive to circulating sex steroid hormones. Clinical and preclinical data suggest that E_2 and P_4 have opposing roles in regulating the rewarding and reinforcing properties of cocaine. For example, exogenous E_2 in ovariectomized female rats enhances operant responding for cocaine self-administration (Larson et al., 2007; Lynch et al., 2001), whereas systemically administered P_4 attenuates responding (Feltenstein et al., 2009). The results presented in this dissertation imply that the mPOA may indeed play this role. Rostral mPOA-to-VTA afferents mainly contain membrane-associated hormone

receptors (GPER and PGRMC1), whereas the central preoptotegmental efferents contain even higher proportions of membrane-associated receptors and a significant amount of ERα (55%). Progesterone receptors were absent from the vast majority of preoptotegmental perikarya. This indicates that E_2, but not P_4, can act by both rapidly changing the electrophysiological properties of the cell (via GPER; Kuo et al., 2010; Roepke, Qiu, Bosch, Rønnekleiv, & Kelly, 2009) and can influence protein translation and membrane permeability in the long term (via ERα; Kuo et al., 2010; McEwen & Alves, 1999; Micevych & Dominguez, 2009). In contrast, the extensive colocalization of PGRMC1 suggests that P_4 may influence the activity of these cells, but its function in regulating cell activity is not fully understood. Indeed, PGRMC1 is more similar to cytochrome-*b*5 heme/steroid binding proteins (Kimura et al., 2012) than it is to G-protein coupled membrane receptors. Further complicating this issue, cocaine also binds to PGRMC1 (Matsumoto, McCracken, Pouw, Zhang, & Bowen, 2002), and has been reported to mediate its psychomotor properties (Lever et al., 2014). PGRMC1-specific agonists also increase DA release in the NAc of freely moving mice (Garcés-Ramírez et al., 2011). Since PGRMC1 has limited expression in the NAc and VTA and is highly expressed in the mPOA (Intlekofer & Petersen, 2011), it is feasible that P_4 may act through preoptotegmental efferents. Without electrophysiological or optogenetic data to scrutinize the specific effects of E_2 or P_4 on these cells, it is difficult to determine how they may influence VTA activity following the activation of hormone receptors. As such, this experiment was mainly exploratory and was intended to give us insight into the possible mechanisms through which E_2 and P_4 may influence preoptotegmental perikarya.

Aside from assessing the subregional neuroanatomy and phenotypic profile of mPOA-to-VTA neurons, we also examined how acute, non-contingent cocaine influenced activity of these cells. In short, the data demonstrated a trend (p = 0.08)

towards cocaine enhancing Fos immunoreactivity, a marker of neuronal activity, in rostral preoptotegmental efferents. To my knowledge, there have only been two studies examining psychostimulant-mediated Fos-ir in preoptotegmental efferents. Colussi-Mas and colleagues (2007) found that acute amphetamine did not significantly increase the percent of preoptotegmental perikarya expressing Fos-ir. The second study done by Mahler and Aston-Jones (2012) was the only study to look at cocaine, but they examined activation of projection neurons from the mPOA to the VTA after cue-induced reinstatement of cocaine and rather than acute cocaine administration, and found no differences. However, both studies did see a notable increase in percent activation of preoptotegmental efferents (e.g., ~7% vs. ~20% in Mahler & Aston Jones, 2012), and any effects were likely masked by stringent post-hoc comparison corrections. Furthermore, neither study examined the rostral mPOA, but focused only on the central region of the mPOA as their region of interest. It may be that preoptotegmental efferents are more sensitive to naturally rewarding stimuli (Numan & Numan, 2003), and the aforementioned studies, including ours, may not have had sufficient statistical power to detect a clear activational effect of psychostimulants. Regardless, our data demonstrate that acute cocaine may increase putative activity in these efferents, but further study is required.

In order to further examine whether the mPOA, as a whole, influence the mesolimbic reward system, we conducted lesion studies to determine mPOA effects on activational, neurochemical, and behavioral responses to acute cocaine. Examining only mPOA-intact female rats first, we reported a significant increase in Fos-ir in the rostral mPOA after acute cocaine. This suggests that the rostral mPOA may influence responsiveness to cocaine. Radiofrequency lesions of the mPOA enhanced cocaine-mediated activity in the shell and core of the NAc and in the VTA as well, albeit to a

lesser extent. The small effect size in the VTA is not surprising, as there are only trace amounts of Fos-ir in the VTA during basal neural activity, and no increase in the animals after acute injections of cocaine compared to saline-treated animals (Zahm et al., 2010). These results suggest that the mPOA, either directly or indirectly, modulates activity in the NAc.

As such, the next experiment evaluated whether the mPOA influences DA release in the NAc in response to cocaine. Microinfusions of neurotoxic levels of NMDA were used to lesion the mPOA, while saline- and cocaine-mediated release of DA and 5-HT was assessed via *in vivo* microdialysis. Locomotion and 5-HT levels were elevated immediately after the cocaine injection, but did not vary between the lesioned and sham-lesioned group. More importantly, female rats with mPOA lesions displayed an enhancement in DA release after cocaine administration compared to the sham-lesioned females. This is the first report to demonstrate that the mPOA is directly involved in regulating cocaine-induced DA release in the NAc. The lack of a drug-by-lesion interaction in locomotion and 5-HT release, along with difference in DA release, suggests that the increase in Fos-ir in lesioned animals as described above is, at least in part, due to an increase in DA release from the VTA.

Finally, moving from protein synthesis and neurochemical events in the NAc to behavioral outputs, the penultimate chapter of this dissertation delineates how the mPOA assists in directing conditioned approach behavior. Using a well-established Pavlovian conditioning paradigm, conditioned place preference, we were able to demonstrate that the mPOA is involved in determining the incentive salience of cocaine. Specifically, lesions of the mPOA significantly increased the difference in time spent in the drug-paired chamber between the pretest and posttest (i.e., drug shift). This was the first experiment to directly assess the influence of the mPOA on conditioned place preference

induced by cocaine alone. Although Periera and Morrell (2010) ascertained that transient inhibition of the mPOA shifted the conditioned placed preference of postpartum female rats from pups to cocaine, but they did not directly assess the effect on the strength of cocaine place preference in isolation. Together, along with several other studies showing the importance of the mPOA in the formation of conditioned place preference to and operant responding for naturally rewarding stimuli (Lee et al., 1999; Morgan et al., 1999; Seip & Morrell, 2007), these experiments suggest that the mPOA is important in regulating the incentive salience of cocaine.

The sum of the data presented above underscores the need to include the mPOA as a secondary node involved in regulating the mesolimbic DA system and associated behavioral outputs. Current reviews of the mesolimbic DA system and its involvement in reward regulation often excludes the mPOA (Carlezon & Thomas, 2009; Koob & Volkow, 2010; Pierce & Kumaresan, 2006; Thomas, Kalivas, & Shaham, 2008). Considering the extent to which it is evolutionary conserved, its similarity in animals throughout the phylogenetic tree (O'Connell & Hofmann, 2012), and its importance in mediating the incentive value of natural and artificial stimuli (Hedges et al., 2010; Pereira & Morrell, 2010), the data support the mPOA as an essential region in regulating reward. As our understanding of functional neuroanatomy involved in regulating incentive salience, reward, and reinforcement is expanded by novel experiments, which includes the addition of other regulatory diencephalic and epithalamic nodes (e.g., Adrover, Shin, & Alvarez, 2014; Nieh, Kim, Namburi, & Tye, 2013; Stuber et al., 2012), we should include the mPOA and a variety of other brain areas to discussions of mesolimbic DA activity.

Additional studies of the role of the mPOA, and hormones in general, on drug reward mechanisms can assist us in understanding the neurobiology of cocaine

acquisition and addiction in women. While the majority of cocaine abusers are male, women still comprise about one-third of users and are in some ways more vulnerable to its addictive and psychomotor properties (Chen & Kandel, 2002; Kosten, Gawin, Kosten, & Rounsaville, 1993). Moreover, preclinical and clinical treatments are usually based on research conducted almost exclusively in males, and do not acknowledge that women respond to cocaine differently throughout their ovulatory cycle (Gillies & Mcarthur, 2010; Lynch et al., 2002). Recent studies of pharmacological and hormonal interventions specifically targeted to female cocaine users, while not always ideal, have shown promise in more effectively curbing cocaine abuse in women (e.g., Sofuoglu et al., 2004).

References

Abrahám, I. M., Todman, M. G., Korach, K. S., & Herbison, A. E. (2004). Critical in vivo roles for classical estrogen receptors in rapid estrogen actions on intracellular signaling in mouse brain. *Endocrinology*, *145*, 3055–3061. doi:10.1210/en.2003-1676

Adamantidis, A. R., Tsai, H.-C., Boutrel, B., Zhang, F., Stuber, G. D., Budygin, E. A., … de Lecea, L. (2011). Optogenetic interrogation of dopaminergic modulation of the multiple phases of reward-seeking behavior. *Journal of Neuroscience*, *31*(30), 10829–10835.

Adrover, M. F., Shin, J. H., & Alvarez, V. A. (2014). Glutamate and dopamine transmission from midbrain dopamine neurons share similar release properties but are differentially affected by cocaine. *Journal of Neuroscience*, *34*(9), 3183–3192. doi:10.1523/JNEUROSCI.4958-13.2014

Afonso, V. M., Mueller, D., Stewart, J., & Pfaus, J. G. (2009). Amphetamine pretreatment facilitates appetitive sexual behaviors in the female rat. *Psychopharmacology*, *205*(1), 35–43. doi:10.1007/s00213-009-1511-x

Ahmed, I. S. A., Chamberlain, C., & Craven, R. J. (2012). S2R(Pgrmc1): the cytochrome-related sigma-2 receptor that regulates lipid and drug metabolism and hormone signaling. *Expert Opinion on Drug Metabolism & Toxicology*, *8*, 361–70. doi:10.1517/17425255.2012.658367

Albin, R. L., Young, A. B., & Penney, J. B. (1989). The functional anatomy of basal ganglia disorders. *Trends in Neurosciences*, *12*(10), 366–375.

Alekseyenko, O. V, Waters, P., Zhou, H., & Baum, M. J. (2007). Bilateral damage to the sexually dimorphic medial preoptic area/anterior hypothalamus of male ferrets causes a female-typical preference for and a hypothalamic Fos response to male body odors. *Physiology & Behavior*, *90*(2-3), 438–49.

Alyea, R. A., Laurence, S. E., Kim, S. H., Katzenellenbogen, B. S., Katzenellenbogen, J. a, & Watson, C. S. (2008). The roles of membrane estrogen receptor subtypes in modulating dopamine transporters in PC-12 cells. *Journal of Neurochemistry*, *106*(4), 1525–33. doi:10.1111/j.1471-4159.2008.05491.x

Anker, J. J., & Carroll, M. E. (2010). Sex differences in the effects of allopregnanolone on yohimbine-induced reinstatement of cocaine seeking in rats. *Drug and Alcohol Dependence*, *107*, 264–7. doi:10.1016/j.drugalcdep.2009.11.002

Baik, J.-H. (2013). Dopamine signaling in reward-related behaviors. *Frontiers in Neural Circuits*, *7*(October), 152. doi:10.3389/fncir.2013.00152

Balfour, M. E., Yu, L., & Coolen, L. M. (2004). Sexual behavior and sex-associated environmental cues activate the mesolimbic system in male rats. *Neuropsychopharmacology*, *29*(4), 718–30. doi:10.1038/sj.npp.1300350

Bali, N., Arimoto, J. M., Iwata, N., Lin, S. W., Zhao, L., Brinton, R. D., … Finch, C. E. (2012). Differential responses of progesterone receptor membrane component-1 (Pgrmc1) and the classical progesterone receptor (Pgr) to 17β-estradiol and progesterone in hippocampal subregions that support synaptic remodeling and neurogenesis. *Endocrinology*, *153*(2), 759–69. doi:10.1210/en.2011-1699

Baliki, M. N., Mansour, A., Baria, A. T., Huang, L., Berger, S. E., Fields, H. L., & Apkarian, A. V. (2013). Parceling Human Accumbens into Putative Core and Shell Dissociates Encoding of Values for Reward and Pain. *Journal of Neuroscience*, *33*(41), 16383–16393. doi:10.1523/JNEUROSCI.1731-13.2013

Balthazart, J., & Ball, G. F. (2007). Topography in the preoptic region: differential regulation of appetitive and consummatory male sexual behaviors. *Frontiers in Neuroendocrinology*, *28*(4), 161–78. doi:10.1016/j.yfrne.2007.05.003

Bardo, M. T., & Bevins, R. A. (2000). Conditioned place preference : what does it add to our preclinical understanding of drug reward? *Psychopharmacologia*, *153*(1), 31–43.

Barker, D. J., Root, D. H., Ma, S., Jha, S., Megehee, L., Pawlak, A. P., & West, M. O. (2010). Dose-dependent differences in short ultrasonic vocalizations emitted by rats during cocaine self-administration. *Psychopharmacology*, *211*(4), 435–42. doi:10.1007/s00213-010-1913-9

Bashour, N. M., & Wray, S. (2012). Progesterone directly and rapidly inhibits GnRH neuronal activity via progesterone receptor membrane component 1. *Endocrinology*, *153*(9), 4457–69. doi:10.1210/en.2012-1122

Bassareo, V., & Di Chiara, G. (1999). Differential responsiveness of dopamine transmission to food-stimuli in nucleus accumbens shell/core compartments. *Neuroscience*, *89*(3), 637–641.

Bazzett, T. J., & Becker, J. B. (1994). Sex differences in the rapid and acute effects of estrogen on striatal D2 dopamine receptor binding. *Brain Research*, *637*(1-2), 163–72.

Beach, F. A., Etkin, W., & Rasquin, P. (1942). Importance of Progesterone to Induction of Sexual Receptivity in Spayed Female Rats. *Experimental Biology and Medicine*, *51*(3), 369–371. doi:10.3181/00379727-51-13979

Beach, F. A., & Jordan, L. (1956). Effects of sexual reinforcement upon the performance of male rats in a straight runway. *Journal of Comparative and Physiological Psychology*, *49*(2), 105–110.

Becker, J. B. (1990). Estrogen rapidly potentiates amphetamine-induced striatal dopamine release and rotational behavior during microdialysis. *Neuroscience Letters*, *118*(2), 169–71.

Becker, J. B. (1999). Gender differences in dopaminergic function in striatum and nucleus accumbens. *Pharmacology Biochemistry and Behavior*, *64*(4), 803–812. doi:10.1016/S0091-3057(99)00168-9

Becker, J. B., & Cha, J. H. (1989). Estrous cycle-dependent variation in amphetamine-induced behaviors and striatal dopamine release assessed with microdialysis. *Behavioural Brain Research*, *35*(2), 117–25.

Becker, J. B., & Hu, M. (2008). Sex differences in drug abuse. *Frontiers in Neuroendocrinology*, *29*(1), 36–47. doi:10.1016/j.yfrne.2007.07.003

Becker, J. B., Molenda, H., & Hummer, D. L. (2001). Gender differences in the behavioral responses to cocaine and amphetamine. Implications for mechanisms mediating gender differences in drug abuse. *Annals of the New York Academy of Sciences*, *937*, 172–87.

Becker, J. B., Perry, A. N., & Westenbroek, C. (2012). Sex differences in the neural mechanisms mediating addiction: a new synthesis and hypothesis. *Biology of Sex Differences*, *3*(1), 14. doi:10.1186/2042-6410-3-14

Becker, J. B., & Rudick, C. N. (1999). Rapid effects of estrogen or progesterone on the amphetamine-induced increase in striatal dopamine are enhanced by estrogen priming : A microdialysis study. *Pharmacology Biochemistry and Behavior*, *64*(1), 53–57.

Been, L. E., & Petrulis, A. (2010). The role of the medial preoptic area in appetitive and consummatory reproductive behaviors depends on sexual experience and odor volatility in male Syrian hamsters. *Neuroscience*, *170*(4), 1120–32. doi:10.1016/j.neuroscience.2010.08.029

Belelli, D., Casula, A., Ling, A., & Lambert, J. J. (2002). The influence of subunit composition on the interaction of neurosteroids with GABA A receptors. *Neuropharmacology*, *43*, 651–661.

Ben-Shahar, O. M., Szumlinski, K. K., Lominac, K. D., Cohen, A., Gordon, E., Ploense, K. L., … Woodward, N. (2013). Extended access to cocaine self-administration results in reduced glutamate function within the medial prefrontal cortex. *Addiction Biology*, *17*(4), 746–757. doi:10.1111/j.1369-1600.2011.00428.x.Extended

Berridge, C. W., & Arnsten, A. F. T. (2013). Psychostimulants and motivated behavior: arousal and cognition. *Neuroscience and Biobehavioral Reviews*, *37*(9), 1976–1984. doi:10.1016/j.neubiorev.2012.11.005

Berridge, K. C., & Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Research Reviews*, *28*(3), 309–369. doi:10.1016/S0165-0173(98)00019-8

Beyer, C., & Gonzalez-mariscal, G. (1989). Lordosis Facilitation in Estrogen Primed Rats by Intrabrain Injection of Pregnanes. *Pharmacology Biochemistry and Behavior*, *31*, 919–926.

Bicego, K. C., Barros, R. C. H., & Branco, L. G. S. (2007). Physiology of temperature regulation: comparative aspects. *Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology*, *147*(3), 616–39. doi:10.1016/j.cbpa.2006.06.032

Björklund, A., & Dunnett, S. B. (2007). Dopamine neuron systems in the brain: an update. *Trends in Neurosciences*, *30*(5), 194–202. doi:10.1016/j.tins.2007.03.006

Blackburn, J. R., Pfaus, J. G., & Phillips, A. G. (1992). Dopamine functions in appetitive and defensive behaviours. *Progress in Neurobiology*, *39*, 247–279.

Blaustein, J. D., & Erskine, M. S. (2002). Female sexual behavior: cellular integration of hormonal and afferent information in the rodent forebrain. In D. W. Pfaff, A. P. Arnold, A. M. Etgen, S. E. Fahrbach, & R. T. Rubin (Eds.), *Hormones, Brain and Behavior* (Vol. 1, pp. 139–214). San Diego: Academic Press.

Blaustein, J. D., Tetel, M. J., Nielsen Ricciardi, K. H., Delville, Y., & Turcotte, J. C. (1994). Hypothalamic ovarian steroid hormone-sensitive neurons involved in female sexual behavior. *Psychoneuroendocrinology*, *19*(5-7), 505–516. doi:10.1016/0306-4530(94)90036-1

Blume, S. B. (1990). Chemical dependency in women: Important issues. *The American Journal of Drug and Alcohol Abuse*, *16*, 297–307.

Bobzean, S. A. M., Dennis, T. S., & Perrotti, L. I. (2014). Acute estradiol treatment affects the expression of cocaine-induced conditioned place preference in ovariectomized female rats. *Brain Research Bulletin*, *103*, 49–53. doi:10.1016/j.brainresbull.2014.02.002

Bobzean, S. A. M., DeNobrega, A. K., & Perrotti, L. I. (2014). Sex differences in the neurobiology of drug addiction. *Experimental Neurology*, *259*, 64–74. doi:10.1016/j.expneurol.2014.01.022

Boling, J. L., & Blandau, R. J. (1939). The estrogen-progesterone induction of mating responses in the spayed female rat. *Endocrinology*, *25*(3), 359–364. doi:10.1210/endo-25-3-359

Boulant, J. A. (1981). Hypothalamic mechanisms in thermoregulation. *Federation Proceedings*, *40*(14), 2843–50.

Bourque, C. W., Oliet, S. H. R., & Richard, D. (1994). Osmoreceptors, osmoreception, and osmoregulation. *Frontiers in Neuroendocrinology*, *15*, 231–274.

Bowman, B. P., Vaughan, S. R., Walker, Q. D., Davis, S. L., Little, P. J., Scheffler, N. M., … Kuhn, C. M. (1999). Effects of sex and gonadectomy on cocaine metabolism in the rat. *The Journal of Pharmacology and Experimental Therapeutics*, *290*(3), 1316–23.

Bowman, E. M., & Richmond, B. J. (1996). Neural signals in the monkey ventral striatum related to motivation for juice and cocaine rewards. *Journal of Neurophysiology*, *75*(3), 1061– 1073.

Brailoiu, E., Dun, S. L., Brailoiu, G. C., Mizuo, K., Sklar, L. A., Oprea, T. I., … Dun, N. J. (2007). Distribution and characterization of estrogen receptor G protein-coupled receptor 30 in the rat central nervous system. *The Journal of Endocrinology*, *193*(2), 311–21. doi:10.1677/JOE-07-0017

Brodie, M. S., & Dunwiddie, T. V. (1990). Cocaine effects in the ventral tegmental area: Evidence for an indirect dopaminergic mechanism of action. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *342*(6), 660–665. doi:10.1007/BF00175709

Browning, J. R., Browning, D. A., Maxwell, A. O., Dong, Y., Jansen, H. T., Panksepp, J., & Sorg, B. A. (2011). Positive affective vocalizations during cocaine and sucrose

self-administration: a model for spontaneous drug desire in rats. *Neuropharmacology*, *61*(1-2), 268–75. doi:10.1016/j.neuropharm.2011.04.012

Bunney, E., Appel, S., & Brodie, M. (2001). Electrophysiological effects of cocaethylene, cocaine, and ethanol on dopaminergic neurons of the ventral tegmental area. *Journal of Pharmacology and Experimental Therapeutics*, *297*(2), 696–703.

Butcher, R. L., Collins, W. E., Fugo, N. W., & Virginia, W. (2013). Plasma concentration of LH, FSH, prolactin, progesterone and estradiol-17β throughout the 4-day estrous cycle of the rat. *Endocrinology*, *94*, 1–2. doi:10.1210/endo-94-6-1704

Cacciapaglia, F., Wightman, R. M., & Carelli, R. M. (2011). Rapid Dopamine Signaling Differentially Modulates Distinct Microcircuits within the Nucleus Accumbens during Sucrose-Directed Behavior. *Journal of Neuroscience*, *31*(39), 13860–13869. doi:10.1523/JNEUROSCI.1340-11.2011

Caggiula, A. R., & Hoebel, B. G. (1966). "Copulation-reward site" in the posterior hypothalamus. *Science*, *153*(3741), 1284–1285. doi:10.1126/science.153.3741.1284

Caine, S. B., Bowen, C. A., Yu, G., Zuzga, D., Negus, S. S., & Mello, N. K. (2004). Effect of gonadectomy and gonadal hormone replacement on cocaine self-administration in female and male rats. *Neuropsychopharmacology*, *29*(5), 929–42.

Cannon, C. M., & Palmiter, R. D. (2003). Reward without Dopamine. *Journal of Neuroscience*, *23*(34), 10827–10831.

Cardona-Gomez, G. P., Mendez, P., & Garcia-Segura, L. M. (2002). Synergistic interaction of estradiol and insulin-like growth factor-I in the activation of PI3K/Akt signaling in the adult rat hypothalamus. *Brain Research. Molecular Brain Research*, *107*(1), 80–8.

Carelli, R. M. (2002). Nucleus accumbens cell firing during goal-directed behaviors for cocaine vs. "natural" reinforcement. *Physiology & Behavior*, *76*(3), 379–387. doi:10.1016/S0031-9384(02)00760-6

Carelli, R. M., & Deadwyler, S. A. (1994). A comparison of nucleus accumbens neuronal firing patterns during cocaine self-administration and water reinforcement in rats. *The Journal of Neuroscience*, *14*(12), 7735–7746.

Carelli, R. M., & Deadwyler, S. A. (1996). Dose-dependent transitions in nucleus accumbens cell firing and behavioral responding during cocaine self-administration

sessions in rats. *The Journal of Pharmacology and Experimental Therapeutics*, *277*(1), 385–93.

Carelli, R. M., & Deadwyler, S. A. (1997). Cellular mechanisms underlying reinforcement-related processing in the nucleus accumbens: behaving animals. *Pharmacology Biochemistry and Behavior*, *57*(3), 495–504. doi:10.1016/S0091-3057(96)00442-X

Carelli, R. M., Ijames, S. G., & Crumling, A. J. (2000). Evidence that separate neural circuits in the nucleus accumbens encode cocaine versus "natural" (water and food) reward. *The Journal of Neuroscience*, *20*(11), 4255–66.

Carlezon, W. A., & Thomas, M. J. (2009). Biological substrates of reward and aversion: a nucleus accumbens activity hypothesis. *Neuropharmacology*, *56 Suppl 1*, 122–32. doi:10.1016/j.neuropharm.2008.06.075

Carlezon, W. A., Devine, D. P., & Wise, R. A. (1995). Habit-forming actions of nomifensine in nucleus accumbens. *Psychopharmacology*, *122*(2), 194–197. doi:10.1007/BF02246095

Carr, G. D., & White, N. M. (1983). Conditioned place preference from intra-accumbens but not intra-caudate amphetamine injections. *Life Sciences*, *33*, 2551–2557.

Carr, G. D., & White, N. M. (1986). Psychopharmacology anatomical disassociation of amphetamine's rewarding and aversive effects : An intracranial microinjection study. *Psychopharmacology*, *89*(I 986), 340–346.

Carrasco, M. A., Jaimovich, E., Kemmerling, U., & Hidalgo, C. (2004). Signal transduction and gene expression regulated by calcium release from internal stores in excitable cells. *Biological Research*, *37*, 701–712. doi:10.4067/S0716-97602004000400028

Carroll, M. E., & Anker, J. J. (2010). Sex differences and ovarian hormones in animal models of drug dependence. *Hormones and Behavior*, *58*, 44–56.

Carroll, M. E., Lynch, W. J., Roth, M. E., Morgan, A. D., & Cosgrove, K. P. (2004). Sex and estrogen influence drug abuse. *Trends in Pharmacological Sciences*, *25*, 273–9. doi:10.1016/j.tips.2004.03.011

Castner, S. A., Xiao, L., & Becker, J. B. (1993). Sex differences in striatal dopamine: in vivo microdialysis and behavioral studies. *Brain Research*, *610*(1), 127–34.

Champagne, F. A., Weaver, I. C. G., Diorio, J., Sharma, S., & Meaney, M. J. (2003). Natural variations in maternal care are associated with estrogen receptor alpha expression and estrogen sensitivity in the medial preoptic area. *Endocrinology*, *144*(11), 4720–4. doi:10.1210/en.2003-0564

Chang, J., Janak, P. H., Woodward, D. J., & Carolina, N. (1998). Comparison of cesocorticolimbic neuronal responses during cocaine and heroin self-administration in freely moving rats. *The Journal of Neuroscience*, *18*(8), 3098–3115.

Chang, J. Y., Sawyer, S. F., Lee, R. S., & Woodward, D. J. (1994). Electrophysiological and pharmacological evidence for the role of the nucleus accumbens in cocaine self-administration in freely moving rats. *The Journal of Neuroscience*, *14*, 1224–44.

Chen, C.-J., & Vandenbergh, J. G. (1994). Effect of chronic cocaine on reproduction in female house mice. *Pharmacology Biochemistry and Behavior*, *48*(4), 909–913. doi:10.1016/0091-3057(94)90199-6

Chen, J. C., Tsai, H.-W., Yeh, K.-Y., Tai, M. Y., & Tsai, Y.-F. (2008). Correlation of catecholamine levels in the bed nucleus of the stria terminalis and reduced sexual behavior in middle-aged male rats. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *63*(7), 678–82.

Chen, K., & Kandel, D. (2002). Relationship between extent of cocaine use and dependence among adolescents and adults in the United States. *Drug and Alcohol Dependence*, *68*(1), 65–85. doi:10.1016/S0376-8716(02)00086-8

Chiba, T., & Murata, Y. (1985). Afferent and efferent connections of the medial preoptic area in the rat: a WGA-HRP study. *Brain Research Bulletin*, *14*(3), 261–72.

Chin, J., Sternin, O., Wu, H. B. K., Burrell, S., Lu, D., Jenab, S., … Quiñones-Jenab, V. (2002). Endogenous gonadal hormones modulate behavioral and neurochemical responses to acute and chronic cocaine administration. *Brain Research*, *945*(1), 123–30.

Cocores, J. A., Dackis, C. A., & Gold, M. S. (1986). Sexual dysfunction secondary to cocaine abuse in two patients. *Journal of Clinical Psychiatry*, *47*(7), 384–385.

Collins, S. L., Evans, S. M., Foltin, R. W., & Haney, M. (2007). Intranasal cocaine in humans: effects of sex and menstrual cycle. *Pharmacology, Biochemistry, and Behavior*, *86*(1), 117–24. doi:10.1016/j.pbb.2006.12.015

Colussi-Mas, J., Geisler, S., Zimmer, L., Zahm, D. S., & Bérod, A. (2007). Activation of afferents to the ventral tegmental area in response to acute amphetamine: a double-

labelling study. *The European Journal of Neuroscience*, *26*(4), 1011–25. doi:10.1111/j.1460-9568.2007.05738.x

Cooke, B. M., & Woolley, C. S. (2005). Sexually dimorphic synaptic organization of the medial amygdala. *The Journal of Neuroscience*, *25*(46), 10759–67. doi:10.1523/JNEUROSCI.2919-05.2005

Cooper, Z. D., Foltin, R. W., & Evans, S. M. (2012). Effects of menstrual cycle phase on cocaine self-administration in rhesus macaques. *Hormones and Behavior*, *63*(1), 105–113. doi:10.1016/j.yhbeh.2012.10.008

Covey, D. P., Roitman, M. F., & Garris, P. A. (2014). Illicit dopamine transients: Reconciling actions of abused drugs. *Trends in Neurosciences*, *37*(4), 200–210. doi:10.1016/j.tins.2014.02.002

Crawford, L. L., Holloway, K. S., & Domjan, M. (1993). The nature of sexual reinforcement. *Journal of the Experimental Analysis of Behavior*, *60*, 55–66.

Cummings, J. A., Jagannathan, L., Jackson, L. R., & Becker, J. B. (2014). Sex differences in the effects of estradiol in the nucleus accumbens and striatum on the response to cocaine: Neurochemistry and behavior. *Drug and Alcohol Dependence*, *135*, 22–8. doi:10.1016/j.drugalcdep.2013.09.009

Dalton, G. L., Phillips, A. G., & Floresco, S. B. (2014). Preferential involvement by nucleus accumbens shell in mediating probabilistic learning and reversal shifts. *The Journal of Neuroscience*, *34*(13), 4618–26. doi:10.1523/JNEUROSCI.5058-13.2014

Damsma, G., Pfaus, J. G., Wenkstern, D., Phillips, A. G., & Fibiger, H. C. (1992). Sexual behavior increases dopamine transmission in the nucleus accumbens and striatum of male Rats : Comparison With novelty and locomotion. *Behavioral Neuroscience*, *106*(1), 181–191.

Deco, G., & Rolls, E. T. (2005). Attention, short-term memory, and action selection: a unifying theory. *Progress in Neurobiology*, *76*(4), 236–56. doi:10.1016/j.pneurobio.2005.08.004

Dennis, M. K., Burai, R., Ramesh, C., Petrie, W. K., Alcon, S. N., Nayak, T. K., … Prossnitz, E. R. (2009). In vivo effects of a GPR30 antagonist. *Nature Chemical Biology*, *5*(6), 421–7. doi:10.1038/nchembio.168

Deutch, A. Y., & Cameron, D. S. (1992). Pharmacological characterization of dopamine systems in th nucleus accumbens core and shell. *Neuroscience*, *46*(1), 49–52.

Di Chiara, G. (2002). Nucleus accumbens shell and core dopamine : differential role in behavior and addiction. *Behavioral and Neural Biology*, *137*, 74–114.

Di Chiara, G., Bassareo, V., Fenu, S., De Luca, M. A., Spina, L., Cadoni, C., … Lecca, D. (2004). Dopamine and drug addiction: the nucleus accumbens shell connection. *Neuropharmacology*, *47 Suppl 1*, 227–41. doi:10.1016/j.neuropharm.2004.06.032

Di Paolo, T. (1994). Modulation of brain dopamine transmission by sex steroids. *Reviews in the Neurosciences*, *5*(1), 27–41.

Dluzen, D. E., & Ramirez, V. D. (1984). Bimodal Effect of Progesterone on in vitro Dopamine Function of the Rat Corpus striatum. *Neuroendocrinology*, *39*(2), 149–155. doi:10.1159/000123971

Dluzen, D. E., & Ramirez, V. D. (1987). Intermittent infusion of progesterone potentiates whereas continuous infusion reduces amphetamine-stimulated dopamine release from ovariectomized estrogen-primed rat striatal fragments superfused in vitro. *Brain Research*, *406*(1-2), 1–9.

Dluzen, D. E., & Ramirez, V. D. (1989). Progesterone effects upon dopamine release from the corpus striatum of female rats. II. Evidence for a membrane site of action and the role of albumin. *Brain Research*, *476*(2), 338–344. doi:10.1016/0006-8993(89)91255-9

Dluzen, D. E., & Ramirez, V. D. (1990). In vitro progesterone modulation of amphetamine-stimulated dopamine release from the corpus striatum of ovariectomized estrogen-treated female rats: response characteristics. *Brain Research*, *517*(1-2), 117–22.

Dluzen, D. E., & Ramirez, V. D. (1991). Modulatory effects of progesterone upon dopamine release from the corpus striatum of ovariectomized estrogen-treated rats are stereo-specific. *Brain Research*, *538*(1), 176–9.

Dominguez, J. M. (2009). A role for preoptic glutamate in the regulation of male reproductive behavior. *The Neuroscientist*, *15*(1), 11–9.

Dominguez, J. M., & Hull, E. M. (2005). Dopamine, the medial preoptic area, and male sexual behavior. *Physiology & Behavior*, *86*(3), 356–368. doi:10.1016/j.physbeh.2005.08.006

DonCarlos, L. L., Monroy, E., & Morrell, J. I. (1991). Distribution of estrogen receptor-immunoreactive cells in the forebrain of the female guinea pig. *The Journal of Comparative Neurology*, *305*, 591–612. doi:10.1002/cne.903050406

Dreher, J.-C., Schmidt, P. J., Kohn, P., Furman, D., Rubinow, D., & Berman, K. F. (2007). Menstrual cycle phase modulates reward-related neural function in women. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(7), 2465–70. doi:10.1073/pnas.0605569104

Dudish, S. A., & Hatsukami, D. K. (1996). Gender differences in crack users who are research volunteers. *Drug and Alcohol Dependence*, *42*, 55–63. doi:10.1016/0376-8716(96)01266-5

Dumitriu, D., Laplant, Q., Grossman, Y. S., Dias, C., Janssen, W. G., Russo, S. J., … Nestler, E. J. (2012). Subregional , dendritic compartment , and spine subtype specificity in cocaine regulation of dendritic spines in the nucleus accumbens. *The Journal of Neuroscience*, *32*(20), 6957–66.

Edlin, B. R., Irwin, K. I., Sairus Faruque, M. P. H., McCoy, C. B., Word, C., Serrano, Y., … Holmberg, S. D. (1994). Intersecting epidemics - crack cocaine use and HIV infection among inner-city young adults. *The New England Journal of Medicine*, *331*(21), 1422–1427.

Edwards, D. A., & Einhorn, L. C. (1986). Preoptic and midbrain control of sexual motivation. *Physiology & Behavior*, *37*(2), 329–35.

Edwards, D. P., Altmann, M., DeMarzo, A., Zhang, Y., Weigel, N. L., & Beck, C. A. (1995). Progesterone receptor and the mechanism of action of progesterone antagonists. *The Journal of Steroid Biochemistry and Molecular Biology*, *53*(1-6), 449–58.

Ehret, G., & Buckenmaier, J. (1995). Estrogen-receptor occurrence in the female mouse brain: Effects of maternal experience, ovariectomy, estrogen and anosmia. *Journal of Physiology*, *88*, 315–329.

Elman, I., Karlsgodt, K. H., & Gastfriend, D. R. (2001). Gender differences in cocaine craving among non-treatment-seeking individuals with cocaine dependence. *The American Journal of Drug and Alcohol Abuse*, *27*(2), 193–202.

Ericson, E., & Ahlenius, S. (1999). Suggestive evidence for inhibitory effects of galanin on mesolimbic dopaminergic neurotransmission. *Brain Research*, *822*(1-2), 200–209. doi:10.1016/S0006-8993(99)01144-0

Evans, S. M. (2007). The role of estradiol and progesterone in modulating the subjective effects of stimulants in humans. *Experimental and Clinical Psychopharmacology*, *15*(5), 418–26.

Evans, S. M., & Foltin, R. W. (2004). Pharmacokinetics of intravenous cocaine across the menstrual cycle in rhesus monkeys. *Neuropsychopharmacology*, *29*(10), 1889–900. doi:10.1038/sj.npp.1300486

Evans, S. M., & Foltin, R. W. (2006). Exogenous progesterone attenuates the subjective effects of smoked cocaine in women, but not in men. *Neuropsychopharmacology*, *31*(3), 659–74.

Evans, S. M., & Foltin, R. W. (2010). Does the response to cocaine differ as a function of sex or hormonal status in human and non-human primates? *Hormones and Behavior*, *58*(1), 13–21. doi:10.1016/j.yhbeh.2009.08.010

Evans, S. M., Haney, M., Fischman, M. W., & Foltin, R. W. (1999). Limited sex differences in response to "binge" smoked cocaine use in humans. *Neuropsychopharmacology*, *21*, 445–454. doi:10.1016/S0893-133X(98)00120-1

Evans, S. M., Haney, M., & Foltin, R. W. (2002). The effects of smoked cocaine during the follicular and luteal phases of the menstrual cycle in women. *Psychopharmacology*, *159*(4), 397–406. doi:10.1007/s00213-001-0944-7

Everitt, B. J. (1990). Sexual motivation : A neural and Behavioural analysis of the mechanisms underlying appetitive and copulatory responses of male rats. *Neuroscience and Biobehavioral Reviews*, *14*(70), 217–232.

Everitt, B. J., & Stacey, P. (1987). Studies of instrumental behavior with sexual reinforcement in male rats (Rattus norvegicus): II. Effects of preoptic area lesions, castration, and testosterone. *Journal of Comparative Psychology*, *101*(4), 407–419. doi:10.1037//0735-7036.101.4.407

Fahrbach, S. E., Morrell, J. I., & Pfaff, D. W. (1986). Identification of medial preoptic neurons that concentrate estradiol and project to the midbrain in the rat. *The Journal of Comparative Neurology*, *247*(3), 364–82.

Fahrbach, S. E., & Pfaff, D. W. (1986). Effect of preoptic region implants of dilute estradiol on the maternal behavior of ovariectomized, nulliparous rats. *Hormones and Behavior*, *20*(3), 354–63.

Fallon, J. H., & Moore, R. Y. (1978). Catecholamine innervation of the basal forebrain. IV. Topography of the dopamine projection to the basal forebrain and neostriatum. *The Journal of Comparative Neurology*, *180*(3), 545–80. doi:10.1002/cne.901800310

Fattore, L., Melis, M., Fadda, P., & Fratta, W. (2014). Sex differences in addictive disorders. *Frontiers in Neuroendocrinology*, *35*(3), 272–84. doi:10.1016/j.yfrne.2014.04.003

Feltenstein, M. W., Byrd, E. A., Henderson, A. R., & See, R. E. (2009). Attenuation of cocaine-seeking by progesterone treatment in female rats. *Psychoneuroendocrinology*, *34*(3), 343–52.

Feltenstein, M. W., & See, R. E. (2007). Plasma progesterone levels and cocaine-seeking in freely cycling female rats across the estrous cycle. *Drug and Alcohol Dependence*, *89*(2-3), 183–9. doi:10.1016/j.drugalcdep.2006.12.017

Fernández-Ruiz, J. J., Amor, J. C., & Ramos, J. A. (1989). Time-dependent effects of estradiol and progesterone on the number of striatal dopaminergic D2-receptors. *Brain Research*, *476*(2), 388–395. doi:10.1016/0006-8993(89)91266-3

Festa, E. D., & Quiñones-Jenab, V. (2004). Gonadal hormones provide the biological basis for sex differences in behavioral responses to cocaine. *Hormones and Behavior*, *46*(5), 509–19.

Festa, E. D., Russo, S. J., Gazi, F. M., Niyomchai, T., Kemen, L. M., Lin, S. N., … Quiñones-Jenab, V. (2004). Sex differences in cocaine-induced behavioral responses, pharmacokinetics, and monoamine levels. *Neuropharmacology*, *46*, 672–687. doi:10.1016/j.neuropharm.2003.11.017

Fibiger, H. C. (1978). Drugs and reinforcement mechanisms: a critical review of the catecholamine theory. *Annual Review of Pharmacology and Toxicology*, *18*, 37–56.

Filardo, E., Quinn, J., Pang, Y., Graeber, C., Shaw, S., Dong, J., & Thomas, P. (2007). Activation of the novel estrogen receptor G protein-coupled receptor 30 (GPR30) at the plasma membrane. *Endocrinology*, *148*(7), 3236–45. doi:10.1210/en.2006-1605

Fleming, A. S., Suh, E. J., Korsmit, M., & Rusak, B. (1994). Activation of Fos-like immunoreactivity in the medial preoptic area and limbic structures of maternal and social interactions in rats. *Behavioral Neuroscience*, *108*(4), 724–734.

Fouriezos, G., Hansson, P., & Wise, R. A. (1978). Neuroleptic-induced attenuation of brain stimulation reward in rats. *Journal of Comparative and Physiological Psychology*, *92*(4), 661–671. doi:10.1037/h0077500

Fox, H. C., Hong, K. A., Paliwal, P., Morgan, P. T., & Sinha, R. (2008). Altered levels of sex and stress steroid hormones assessed daily over a 28-day cycle in early abstinent

cocaine-dependent females. *Psychopharmacology*, *195*(4), 527–36. doi:10.1007/s00213-007-0936-3

Francis, K., Meddle, S. L., Bishop, V. R., & Russell, J. A. (2002). Progesterone receptor expression in the pregnant and parturient rat hypothalamus and brainstem. *Brain Research*, *927*(1), 18–26. doi:10.1016/S0006-8993(01)03318-2

Frye, C. A. (2007). Progestins influence motivation, reward, conditioning, stress, and/or response to drugs of abuse. *Pharmacology, Biochemistry, and Behavior*, *86*(2), 209–19.

Frye, C. A., & Rhodes, M. E. (2006). Administration of estrogen to ovariectomized rats promotes conditioned place preference and produces moderate levels of estrogen in the nucleus accumbens. *Brain Research*, *1067*(1), 209–15. doi:10.1016/j.brainres.2005.10.038

Frye, C. A., Walf, A. A., Kohtz, A. S., & Zhu, Y. (2013). Membrane progestin receptors in the midbrain ventral tegmental area are required for progesterone-facilitated lordosis of rats. *Hormones and Behavior*, *64*(3), 539–45. doi:10.1016/j.yhbeh.2013.05.012

Garcés-Ramírez, L., Green, J. L., Hiranita, T., Kopajtic, T. A., Mereu, M., Thomas, A. M., … Tanda, G. (2011). Sigma receptor agonists : Receptor binding and effects on mesolimbic dopamine neurotransmission assessed by microdialysis. *Biological Psychiatry*, *69*(3), 208–217. doi:10.1016/j.biopsych.2010.07.026

Geisler, S., Derst, C., Veh, R. W., & Zahm, D. S. (2007). Glutamatergic afferents of the ventral tegmental area in the rat. *The Journal of Neuroscience*, *27*(21), 5730–43. doi:10.1523/JNEUROSCI.0012-07.2007

Gerfen, C. R., Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J., & Sibley, D. R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science*, *250*(4986), 1429–32.

Gillies, G. E., & Mcarthur, S. (2010). Estrogen actions in the brain and the basis for differential action in men and momen : A case for sex-specific medicines. *Pharmacological Reviews*, *62*(2), 155–198. doi:10.1124/pr.109.002071.155

Goodman, A. L., Descalzi, C. D., Johnson, D. K., & Hodgen, G. D. (1977). Composite pattern of circulating LH, FSH, estradiol, and progesterone during the menstrual cycle in cynomolgus monkeys. *Experimental Biology and Medicine*, *155*(4), 479–481. doi:10.3181/00379727-155-39834

Gordon, A., Aguilar, R., Garrido-Gracia, J. C., Guil-Luna, S., Sánchez-Cespedes, R., Millán, Y., … Sánchez-Criado, J. E. (2009). Activation of estrogen receptor-alpha induces gonadotroph progesterone receptor expression and action differently in young and middle-aged ovariectomized rats. *Human Reproduction*, *24*(10), 2618–28. doi:10.1093/humrep/dep237

Gorski, R. A., Gordon, J. H., Shryne, J. E., Southam, A. M., & Angeles, L. (1978). Evidence for a morphological sex difference within the medial preoptic area of the rat brain. *Brain Research*, *148*, 333–346.

Gorski, R. A., Harlan, R. E., Jacobson, C. D., Shryne, J. E., & Southam, A. M. (1980). Evidence for the existence of a sexually dimorphic nucleus in the preoptic area of the rat. *The Journal of Comparative Neurology*, *193*(2), 529–39. doi:10.1002/cne.901930214

Gouzoulis-Mayfrank, E., & Daumann, J. (2006). The confounding problem of polydrug use in recreational ecstasy/MDMA users: a brief overview. *Journal of Psychopharmacology*, *20*, 188–193. doi:10.1177/0269881106059939

Grace, A. A., Floresco, S. B., Goto, Y., & Lodge, D. J. (2007). Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. *Trends in Neurosciences*, *30*(5), 220–7. doi:10.1016/j.tins.2007.03.003

Graham, M. D., & Pfaus, J. G. (2010). Differential regulation of female sexual behaviour by dopamine agonists in the medial preoptic area. *Pharmacology, Biochemistry, and Behavior*, *97*(2), 284–92. doi:10.1016/j.pbb.2010.08.012

Graham, M. D., & Pfaus, J. G. (2012). Differential effects of dopamine antagonists infused to the medial preoptic area on the sexual behavior of female rats primed with estrogen and progesterone. *Pharmacology, Biochemistry, and Behavior*, *102*(4), 532–539. doi:10.1016/j.pbb.2012.06.020

Graves, N. S., Hayes, H., Fan, L., & Curtis, K. S. (2011). Time course of behavioral, physiological, and morphological changes after estradiol treatment of ovariectomized rats. *Physiology & Behavior*, *103*(3-4), 261–7. doi:10.1016/j.physbeh.2011.02.017

Graybiel, A. M., Moratalla, R., & Robertson, H. A. (1990). Amphetamine and cocaine induce drug-specific activation of the c-fos gene in striosome-matrix compartments and limbic subdivisions of the striatum. *Proceedings of the National Academy of Sciences of the United States of America*, *87*(17), 6912–6.

Green, C. A. (2006). Gender and use of substance abuse treatment services. *Alcohol Research & Health*, *29*, 55–62.

Greenfield, S., Manwani, S., & Nargiso, J. (2003). Epidemiology of substance use disorders in women. *Obstetrics and Gynecology Clinics of North America*, *30*(3), 413–446. doi:10.1016/S0889-8545(03)00072-X

Griffin, M. L., Weiss, R. D., Mirin, S. M., & Lange, U. (1989). A comparison of male and female cocaine abusers. *Archives of General Psychiatry*, *46*(2), 122–126.

Guarraci, F. A., & Clark, A. S. (2006). Ibotenic acid lesions of the medial preoptic area disrupt the expression of partner preference in sexually receptive female rats. *Brain Research*, *1076*(1), 163–70. doi:10.1016/j.brainres.2005.12.120

Haas, A. L., & Peters, R. H. (2000). Development of substance abuse problems among drug-involved offenders: Evidence for the telescoping effect. *Journal of Substance Abuse*, *12*, 241–253.

Hagihara, K., Hirata, S., Osada, T., Hirai, M., & Kato, J. (1992). Distribution of cells containing progesterone receptor mRNA in the female rat di- and telencephalon: an in situ hybridization study. *Molecular Brain Research*, *14*, 239–249.

Hansen, S. (1994). Maternal behavior of female rats with 6-OHDA lesions in the ventral striatum: Characterization of the pup retrieval deficit. *Physiology & Behavior*, *55*(4), 615–620.

Hansen, S., Bergvall, Å. H., & Nyiredi, S. (1993). Interaction with pups enhances dopamine release in the ventral striatum of maternal rats: A microdialysis study. *Pharmacology Biochemistry and Behavior*, *45*(3), 673–676. doi:10.1016/0091-3057(93)90523-V

Hansen, S., Harthon, C., Wallin, E., Lofberg, L., & Svensson, K. (1991). The effects of 6-OHDA-induced dopamine depletions in the ventral or dorsal striatum on maternal and sexual behavior in the female rat. *Pharmacology Biochemistry and Behavior*, *39*, 71–77.

Hart, A. S., Rutledge, R. B., Glimcher, P. W., & Phillips, P. E. M. (2014). Phasic dopamine release in the rat nucleus accumbens symmetrically encodes a reward prediction error term. *Journal of Neuroscience*, *34*(3), 698–704. doi:10.1523/JNEUROSCI.2489-13.2014

Hazell, G. G. J., Yao, S. T., Roper, J. A., Prossnitz, E. R., O'Carroll, A.-M., & Lolait, S. J. (2009). Localisation of GPR30, a novel G protein-coupled oestrogen receptor,

suggests multiple functions in rodent brain and peripheral tissues. *The Journal of Endocrinology*, *202*, 223–36. doi:10.1677/JOE-09-0066

Hedges, V. L., Staffend, N. A., & Meisel, R. L. (2010). Neural mechanisms of reproduction in females as a predisposing factor for drug addiction. *Current Protocols in Neuroscience*, *31*(2), 217–31. doi:10.1016/j.yfrne.2010.02.003

Heimer, L., Zahm, D. S., Churchill, L., Kalivas, P. W., & Wohltmann, C. (1991). Specificity in the projection patterns of accumbal core and shell in the rat. *Neuroscience*, *41*, 89–125.

Heldring, N., Pike, A., Andersson, S., Matthews, J., Cheng, G., Treuter, E., … Gustafsson, J.-Å. (2007). Estrogen receptors: how do they signal and what are their targets. *Physiological Review*, *87*, 905–931. doi:10.1152/physrev.00026.2006.

Herbison, A. E., Augood, S. J., & McGowan, E. M. (1992). Expression of glutamic acid decarboxylase messenger RNA in rat medial preoptic area neurones during the oestrous cycle and after ovariectomy. *Molecular Brain Research*, *14*(4), 310–6.

Herbison, A. E., Augood, S. J., Simonian, S. X., & Chapman, C. (1995). Regulation of GABA transporter activity and mRNA expression by estrogen in rat preoptic area. *The Journal of Neuroscience*, *15*(12), 8302–8309.

Herbison, A. E., & Fenelon, V. S. (1995). Estrogen Regulation of GABA , Receptor Subunit mRNA Expression in Preoptic Area and Bed Nucleus of the Stria Terminalis of Female Rat Brain. *The Journal of Neuroscience*, *15*(3), 2328–2337.

Herman, J. P., & Cullinan, W. E. (1997). Neurocircuitry of stress: central control of the hypothalamo-pituitary-adrenocortical axis. *Trends in Neurosciences*, *20*(2), 78–84.

Hernández-González, M., Navarro-Meza, M., Prieto-Beracoechea, C. A., & Guevara, M. A. (2005). Electrical activity of prefrontal cortex and ventral tegmental area during rat maternal behavior. *Behavioural Processes*, *70*(2), 132–43. doi:10.1016/j.beproc.2005.06.002

Herndon, J. G. (1976). Effects of midbrain lesions on female sexual behavior in the rat. *Physiology & Behavior*, *17*, 143–148.

Hoebel, B. G. (1997). Neuroscience and appetitive behavior research: 25 years. *Appetite*, *29*(2), 119–33. doi:10.1006/appe.1997.0126

Hoebel, B. G., Hern, L., Aulisi, E. F., Stanley, B. G., & Lenard, L. (1983). Self-injection of amphetamine directly into the brain. *Psychopharmacology*, *81*, 158–163.

Holmes, N. M., & Fam, J. (2013). How does dopamine release in the nucleus accumbens core relate to encoding of a Pavlovian incentive stimulus? *The Journal of Neuroscience*, *33*(25), 10191–2. doi:10.1523/JNEUROSCI.1543-13.2013

Hong, S. (2011). Dopamine-mediated learning and switching in cortico-striatal circuit explain behavioral changes in reinforcement learning. *Frontiers in Behavioral Neuroscience*, *5*(March), 1–17. doi:10.3389/fnbeh.2011.00015

Hu, M., & Becker, J. B. (2003). Effects of sex and estrogen on behavioral sensitization to cocaine in rats. *The Journal of Neuroscience*, *23*(2), 693–9.

Hu, M., & Becker, J. B. (2008). Acquisition of cocaine self-administration in ovariectomized female rats: effect of estradiol dose or chronic estradiol administration. *Drug and Alcohol Dependence*, *94*(1-3), 56–62. doi:10.1016/j.drugalcdep.2007.10.005

Hu, M., Crombag, H. S., Robinson, T. E., & Becker, J. B. (2004). Biological basis of sex differences in the propensity to self-administer cocaine. *Neuropsychopharmacology*, *29*(1), 81–5. doi:10.1038/sj.npp.1300301

Hudson, A., & Stamp, J. A. (2011). Ovarian hormones and propensity to drug relapse: a review. *Neuroscience and Biobehavioral Reviews*, *35*(3), 427–36. doi:10.1016/j.neubiorev.2010.05.001

Hull, E. M. (2011). Sex, drugs and gluttony: How the brain controls motivated behaviors. *Physiology & Behavior*, *104*(1), 173–177.

Hull, E. M., & Dominguez, J. M. (2007). Sexual behavior in male rodents. *Hormones and Behavior*, *52*(1), 45–55. doi:10.1016/j.yhbeh.2007.03.030

Hull, E. M., Du, J., Lorrain, D. S., & Matuszewich, L. (1995). Extracellular dopamine in the medial preoptic area: implications for sexual motivation and hormonal control of copulation. *The Journal of Neuroscience*, *15*(11), 7465–71.

Hull, E. M., Lorrain, D. S., Du, J., Matuszewich, L., Lumley, L. A., Putnam, S. K., & Moses, J. (1999). Hormone-neurotransmitter interactions in the control of sexual behavior. *Behavioral Brain Research*, *105*(1), 105–16.

Hull, E. M., & Rodriguez-Manzo, G. (2009). Male sexual behavior. In D. W. Pfaff, A. P. Arnold, A. M. Etgen, S. E. Fahrbach, & R. T. Rubin (Eds.), *Hormones, Brain and Behavior* (2nd ed., Vol. 1, pp. 5–65). San Diego: Elsevier.

Ikemoto, S. (2007). Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain Research Reviews*, *56*(1), 27–78. doi:10.1016/j.brainresrev.2007.05.004

Ikemoto, S., & Bonci, A. (2014). Neurocircuitry of drug reward. *Neuropharmacology*, *76*, 329–341. doi:10.1016/j.neuropharm.2013.04.031

Ikemoto, S., & Glazier, B. S. (1997). Role of dopamine D1 and D2 receptors in the nucleus accumbens in mediating reward. *The Journal of Neuroscience*, *17*(21), 8580–8587.

Ikemoto, S., Kohl, R. R., & McBride, W. J. (1997). $GABA_A$ receptor blockade in the anterior ventral tegmental area increases extracellular levels of dopamine in the nucleus accumbens of rats. *Journal of Neurochemistry*, *69*(1), 137–143. doi:10.1046/j.1471-4159.1997.69010137.x

Ikemoto, S., Murphy, J. M., & McBride, W. J. (1997). Self-infusion of GABA(A) antagonists directly into the ventral tegmental area and adjacent regions. *Behavioral Neuroscience*, *111*(2), 369–380.

Ikemoto, S., & Panksepp, J. (1996). Dissociations between appetitive and consummatory responses by pharmacological manipulations of reward-relevant brain regions. *Behavioral Neuroscience*, *110*(2), 331–345. doi:10.1037//0735-7044.110.2.331

Ikemoto, S., & Panksepp, J. (1999). The role of nucleus accumbens dopamine in motivated behavior: a unifying interpretation with special reference to reward-seeking. *Brain Research Reviews*, *31*(1), 6–41. doi:10.1016/S0165-0173(99)00023-5

Ilango, A., Kesner, A. J., Broker, C. J., Wang, D. V, & Ikemoto, S. (2014). Phasic excitation of ventral tegmental dopamine neurons potentiates the initiation of conditioned approach behavior: parametric and reinforcement-schedule analyses. *Frontiers in Behavioral Neuroscience*, *8*(May), 155. doi:10.3389/fnbeh.2014.00155

Intlekofer, K. A., & Petersen, S. L. (2011a). 17β-estradiol and progesterone regulate multiple progestin signaling molecules in the anteroventral periventricular nucleus, ventromedial nucleus and sexually dimorphic nucleus of the preoptic area in female rats. *Neuroscience*, *176*, 86–92. doi:10.1016/j.neuroscience.2010.12.033

Intlekofer, K. A., & Petersen, S. L. (2011b). Distribution of mRNAs encoding classical progestin receptor, progesterone membrane components 1 and 2, serpine mRNA binding protein 1, and progestin and ADIPOQ receptor family members 7 and 8 in rat forebrain. *Neuroscience*, *172*, 55–65. doi:10.1016/j.neuroscience.2010.10.051

Ito, R., Robbins, T. W., & Everitt, B. J. (2004). Differential control over cocaine-seeking behavior by nucleus accumbens core and shell. *Nature Neuroscience*, *7*(4), 389–97. doi:10.1038/nn1217

Jackson, L. R., Robinson, T. E., & Becker, J. B. (2006). Sex differences and hormonal influences on acquisition of cocaine self-administration in rats. *Neuropsychopharmacology*, *31*(1), 129–38.

Jalabert, M., Aston-Jones, G. S., Herzog, E., Manzoni, O., & Georges, F. (2009). Role of the bed nucleus of the stria terminalis in the control of ventral tegmental area dopamine neurons. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, *33*(8), 1336–46. doi:10.1016/j.pnpbp.2009.07.010

Jeffcoat, A. R., Perez-Reyes, M., Hill, J. M., Sadler, B. M., & Cook, C. E. (1989). Cocaine disposition in humans after intravenous injection, nasal insufflation (snorting), or smoking. *Drug Metabolism and Disposition*, *17*(2).

Jenkins, W. J., & Becker, J. B. (2003). Female rats develop conditioned place preferences for sex at their preferred interval. *Hormones and Behavior*, *43*(4), 503–507. doi:10.1016/S0018-506X(03)00031-X

Jhou, T. C., Fields, H. L., Baxter, M. G., Saper, C. B., & Holland, P. C. (2009). The rostromedial tegmental nucleus (RMTg), a GABAergic afferent to midbrain dopamine neurons, encodes aversive stimuli and inhibits motor responses. *Neuron*, *61*(5), 786–800. doi:10.1016/j.neuron.2009.02.001

Jhou, T. C., Good, C. H., Rowley, C. S., Xu, S., Wang, H., Burnham, N. W., … Carolina, S. (2013). Cocaine drives aversive conditioning via delayed activation of dopamine-responsive habenular and midbrain pathways. *The Journal of Neuroscience*, *33*(17), 7501–7512. doi:10.1523/JNEUROSCI.3634-12.2013

Johnson, S. W., & North, R. A. (1992). Two types of neurone in the rat ventral tegmental area and their synaptic inputs. *Journal of Physiology*, *450*, 455–468.

Kalivas, P. W., & Duffy, P. (1990). Effect of acute and daily cocaine treatment on extracellular dopamine in the nucleus accumbens. *Synapse*, *5*(1), 48–58. doi:10.1002/syn.890050104

Kalivas, P. W., Widerlov, E., Stanley, D., Breese, G., & Prange, A. J. (1983). Enkephalin dependent action on the mesolimbic system : A dopamine- and a dopamine-independent increase in locomotor. *The Journal of Pharmacology and Experimental Therapeutics*, *227*(1), 229–237.

Ke, F.-C., & Ramirez, V. D. (1990). Binding of progesterone to nerve cell membranes of rat brain using progesterone conjugated to 125 I-bovine serum albumin as a ligand. *Journal of Neurochemistry*, *54*(2), 467–472. doi:10.1111/j.1471-4159.1990.tb01895.x

Keer, S. E., & Stern, J. M. (1999). Dopamine receptor blockade in the nucleus accumbens inhibits maternal retrieval and licking, but enhances nursing behavior in lactating rats. *Physiology & Behavior*, *67*(5), 659–669. doi:10.1016/S0031-9384(99)00116-X

Kelley, A. E. (2006). Worms clear the smoke surrounding nicotine addiction. *Cell*, *127*(3), 460–2. doi:10.1016/j.cell.2006.10.024

Kelley, A. E., & Berridge, K. C. (2002). The neuroscience of natural rewards: relevance to addictive drugs. *The Journal of Neuroscience*, *22*(9), 3306–11. doi:20026361

Kelley, A. E., & Stinus, L. (1985). Disappearance of hoarding behavior after 6-hydroxydopamine lesions of the mesolimbic dopamine neurons and its reinstatement with l-dopa. *Behavioral Neuroscience*, *99*(3), 531–545. doi:10.1037//0735-7044.99.3.531

Kelley, A. E., & Swanson, C. J. (1997). Feeding induced by blockade of AMPA and kainate receptors within the ventral striatum: a microinfusion mapping study. *Behavioural Brain Research*, *89*(1-2), 107–113. doi:10.1016/S0166-4328(97)00054-5

Kelly, M. J., Moss, R. L., Dudley, C. A., & Fawcett, C. P. (1977). The specificity of the response of preoptic-septal area neurons to estrogen : 17α-estradiol versus 17β-estradiol and the response of extrahypothalamic neurons. *Experimental Brain Research*, *52*, 43–52.

Kelm, M. K., Criswell, H. E., & Breese, G. R. (2011). Ethanol-enhanced GABA release: A focus on G protein-coupled receptors. *Brain Research Reviews*, *65*, 113–123. doi:10.1016/j.brainresrev.2010.09.003

Kerstetter, K. A., Aguilar, V. R., Parrish, A. B., & Kippin, T. E. (2008). Protracted time-dependent increases in cocaine-seeking behavior during cocaine withdrawal in female relative to male rats. *Psychopharmacology*, *198*(1), 63–75. doi:10.1007/s00213-008-1089-8

Kerstetter, K. A., Ballis, M. A., Duffin-Lutgen, S., Carr, A. E., Behrens, A. M., & Kippin, T. E. (2012). Sex differences in selecting between food and cocaine reinforcement are mediated by estrogen. *Neuropsychopharmacology*, *37*(12), 2605–14. doi:10.1038/npp.2012.99

Kest, K., Cruz, I., Chen, D. H., Galaj, E., & Ranaldi, R. (2012). A food-associated CS activates c-Fos in VTA DA neurons and elicits conditioned approach. *Behavioural Brain Research*, *235*(2), 150–7. doi:10.1016/j.bbr.2012.07.044

Khan, Z. U., Gutiérrez, A., Martín, R., Peñafiel, A., Rivera, A., & De La Calle, A. (1998). Differential regional and cellular distribution of dopamine D2-like receptors: an immunocytochemical study of subtype-specific antibodies in rat and human brain. *The Journal of Comparative Neurology*, *402*(3), 353–71.

Kimura, I., Nakayama, Y., Konishi, M., Terasawa, K., Ohta, M., Itoh, N., & Fujimoto, M. (2012). Functions of MAPR (Membrane-Associated Progesterone Receptor) family members as heme/steroid-binding proteins. *Current Protein & Peptide Science*, *13*(7), 687–696. doi:10.2174/138920312804142110

Kippin, T. E., Fuchs, R. A., Mehta, R. H., Case, J. M., Parker, M. P., Bimonte-Nelson, H. A., & See, R. E. (2005). Potentiation of cocaine-primed reinstatement of drug seeking in female rats during estrus. *Psychopharmacology*, *182*(2), 245–52. doi:10.1007/s00213-005-0071-y

Kohlert, J. G., & Meisel, R. L. (1999). Sexual experience sensitizes mating-related nucleus accumbens dopamine responses of female Syrian hamsters. *Behavioural Brain Research*, *99*(1), 45–52. doi:10.1016/S0166-4328(98)00068-0

Koob, G. F., & Le Moal, M. (2001). Drug addiction , dysregulation of reward , and allostasis. *Neuropsychopharmacology*, *24*(2), 97–129.

Koob, G. F., Riley, S. J., Smith, S. C., & Robbins, T. W. (1978). Effects of 6-hydroxydopamine lesions of the nucleus accumbens septi and olfactory tubercle on feeding, locomotor activity, and amphetamine anorexia in the rat. *Journal of Comparative and Physiological Psychology*, *92*(5), 917–927. doi:10.1037/h0077542

Koob, G. F., Sanna, P. P., & Bloom, F. E. (1998). Neuroscience of addiction. *Neuron*, *21*, 467–476.

Koob, G. F., & Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology*, *35*(1), 217–38.

Kosten, T. A., Gawin, F. H., Kosten, T. R., & Rounsaville, B. J. (1993). Gender differences in cocaine use and treatment response. *Journal of Substance Abuse Treatment*, *10*, 63–66. doi:10.1016/0740-5472(93)90100-G

Kosten, T. R., McDougle, C. J., Hameedi, F. A., McCance, E. F., Rosen, M. I., Oliveto, A. H., & Price, L. H. (1996). Gender differences in response to intranasal cocaine

administration to humans. *Biological Psychiatry*, *39*, 147–8. doi:10.1016/0006-3223(95)00386-X

Krebs, C. J., Jarvis, E. D., Chan, J., Lydon, J. P., Ogawa, S., & Pfaff, D. W. (2000). A membrane-associated progesterone-binding protein, 25-Dx, is regulated by progesterone in brain regions involved in female reproductive behaviors. *Proceedings of the National Academy of Sciences of the United States of America*, *97*(23), 12816–21. doi:10.1073/pnas.97.23.12816

Kudo, T., Uchigashima, M., Miyazaki, T., Konno, K., Yamasaki, M., Yanagawa, Y., … Watanabe, M. (2012). Three types of neurochemical projection from the bed nucleus of the stria terminalis to the ventral tegmental area in adult mice. *Journal of Neuroscience*, *32*(50), 18035–18046. doi:10.1523/JNEUROSCI.4057-12.2012

Kufahl, P. R., Li, Z., Risinger, R. C., Rainey, C. J., Wu, G., Bloom, A. S., & Li, S.-J. (2005). Neural responses to acute cocaine administration in the human brain detected by fMRI. *NeuroImage*, *28*(4), 904–14. doi:10.1016/j.neuroimage.2005.06.039

Kufahl, P. R., Zavala, A. R., Singh, A., Thiel, K. J., Dickey, E. D., Joyce, J. N., & Neisewander, J. L. (2009). c-Fos expression associated with reinstatement of cocaine-seeking behavior by response-contingent conditioned cues. *Synapse*, *835*(September 2008), 823–835.

Kuhar, M. J., Ritz, M. C., & Boja, J. W. (1991). The dopamine hypothesis of the reinforcing properties of cocaine. *Trends in Neurosciences*, *14*(7), 299–302. doi:10.1016/0166-2236(91)90141-G

Kumar, V. M., Vetrivelan, R., & Mallick, H. N. (2007). Noradrenergic afferents and receptors in the medial preoptic area: neuroanatomical and neurochemical links between the regulation of sleep and body temperature. *Neurochemistry International*, *50*, 783–90. doi:10.1016/j.neuint.2007.02.004

Kuo, J., Hamid, N., Bondar, G., Prossnitz, E. R., & Micevych, P. (2010). Membrane estrogen receptors stimulate intracellular calcium release and progesterone synthesis in hypothalamic astrocytes. *The Journal of Neuroscience*, *30*(39), 12950–7.

Larson, E. B., Anker, J. J., Gliddon, L. A., Fons, K. S., & Carroll, M. E. (2007). Effects of estrogen and progesterone on the escalation of cocaine self-administration in female rats during extended access. *Experimental and Clinical Psychopharmacology*, *15*(5), 461–471.

Laviolette, S. R., & van der Kooy, D. (2001). GABAA receptors in the ventral tegmental area control bidirectional reward signalling between dopaminergic and non-dopaminergic neural motivational systems. *European Journal of Neuroscience*, *13*(5), 1009–1015. doi:10.1046/j.1460-9568.2001.01458.x

Leavitt, F. I. (1969). Drug-induced modifications in sexual behavior and open field locomotion of male rats. *Physiology & Behavior*, *4*, 677–683.

Lee, A., Clancy, S., & Fleming, A. S. (1999). Mother rats bar-press for pups: effects of lesions of the mpoa and limbic sites on maternal behavior and operant responding for pup-reinforcement. *Behavioural Brain Research*, *100*(1-2), 15–31. doi:10.1016/S0166-4328(98)00109-0

Leibowitz, S. F., Akabayashi, A., Wang, J., Alexander, J. T., Dourmashkin, J. T., & Chang, G.-Q. (2007). Increased caloric intake on a fat-rich diet: role of ovarian steroids and galanin in the medial preoptic and paraventricular nuclei and anterior pituitary of female rats. *Journal of Neuroendocrinology*, *19*(10), 753–66. doi:10.1111/j.1365-2826.2007.01584.x

Lever, J. R., Miller, D. K., Green, C. L., Fergason-Cantrell, E. A., Watkinson, L. D., Carmack, T. L., … Lever, S. Z. (2014). A selective sigma-2 receptor ligand antagonizes cocaine-induced hyperlocomotion in mice. *Synapse*, *68*(2), 73–84. doi:10.1002/syn.21717

Lévesque, D., & Di Paolo, T. (1991). Dopamine receptor reappearance after irreversible receptor blockade: effect of chronic estradiol treatment of ovariectomized rats. *Molecular Pharmacology*, *39*, 659–665.

Levin, R. J. (2014). The pharmacology of the human female orgasm- its biological and physiological backgrounds. *Pharmacology, Biochemistry, and Behavior*, *121*, 62–70. doi:10.1016/j.pbb.2014.02.010

Li, M., & Fleming, A. S. (2003). The nucleus accumbens shell is critical for normal expression of pup-retrieval in postpartum female rats. *Behavioural Brain Research*, *145*(1-2), 99–111. doi:10.1016/S0166-4328(03)00135-9

Liu, B., & Arbogast, L. A. (2009). Gene expression profiles of intracellular and membrane progesterone receptor isoforms in the mediobasal hypothalamus during pro-oestrus. *Journal of Neuroendocrinology*, *21*(12), 993–1000. doi:10.1111/j.1365-2826.2009.01920.x

Liu, Y. C., Salamone, J. D., & Sachs, B. D. (1997). Lesions in medial preoptic area and bed nucleus of stria terminalis: differential effects on copulatory behavior and noncontact erection in male rats. *The Journal of Neuroscience*, *17*(13), 5245–53.

Liu, Y.-C., Sachs, B. D., & Salamone, J. D. (1998). Sexual behavior in male rats after radiofrequency or dopamine-depleting lesions in nucleus accumbens. *Pharmacology Biochemistry and Behavior*, *60*(2), 585–592.

Liu, Z.-H., Shin, R., & Ikemoto, S. (2008). Dual role of medial A10 dopamine neurons in affective encoding. *Neuropsychopharmacology*, *33*(12), 3010–20. doi:10.1038/npp.2008.4

Lonstein, J. S. (2002). Frank A. Beach Award: Parenting--the other reproductive behavior. *Hormones and Behavior*, *42*(3), 258–62.

Lonstein, J. S., & De Vries, G. J. (2000). Maternal behaviour in lactating rats stimulates c-fos in glutamate decarboxylase-synthesizing neurons of the medial preoptic area, ventral bed nucleus of the stria terminalis, and ventrocaudal periaqueductal gray. *Neuroscience*, *100*(3), 557–68.

Lonstein, J. S., Dominguez, J. M., Putnam, S. K., De Vries, G. J., & Hull, E. M. (2003). Intracellular preoptic and striatal monoamines in pregnant and lactating rats: possible role in maternal behavior. *Brain Research*, *970*(1-2), 149–58.

Lu, X., Ghasemzadeh, M. B., & Kalivas, P. W. (1998). Expression of D1 receptor, D2 receptor, substance P and enkephalin messenger RNAs in the neurons projecting from the nucleus accumbens. *Neuroscience*, *82*(3), 767–780.

Lukas, S. E., Sholar, M., Lundahl, L. H., Lamas, X., Kouri, E., Wines, J. D., … Mendelson, J. H. (1996). Sex differences in plasma cocaine levels and subjective effects after acute cocaine administration in human volunteers. *Psychopharmacology*, *125*(4), 346–54.

Lüscher, C., & Malenka, R. C. (2011). Drug-evoked synaptic plasticity in addiction: from molecular changes to circuit remodeling. *Neuron*, *69*(4), 650–63. doi:10.1016/j.neuron.2011.01.017

Lynch, W. J. (2008). Acquisition and maintenance of cocaine self-administration in adolescent rats: effects of sex and gonadal hormones. *Psychopharmacology*, *197*, 237–46. doi:10.1007/s00213-007-1028-0

Lynch, W. J., Arizzi, M. N., & Carroll, M. E. (2000). Effects of sex and the estrous cycle on regulation of intravenously self-administered cocaine in rats. *Psychopharmacology*, *152*(2), 132–139.

Lynch, W. J., & Carroll, M. E. (1999). Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. *Psychopharmacology*, *144*, 77–82.

Lynch, W. J., & Carroll, M. E. (2000). Reinstatement of cocaine self-administration in rats: sex differences. *Psychopharmacology*, *148*, 196–200.

Lynch, W. J., Roth, M. E., & Carroll, M. E. (2002). Biological basis of sex differences in drug abuse: preclinical and clinical studies. *Psychopharmacology*, *164*, 121–37.

Lynch, W. J., Roth, M. E., Mickelberg, J. L., & Carroll, M. E. (2001). Role of estrogen in the acquisition of intravenously self-administered cocaine in female rats. *Pharmacology, Biochemistry, and Behavior*, *68*, 641–6.

Lynch, W. J., Sughondhabirom, A., Pittman, B., Gueorguieva, R., Kalayasiri, R., Joshua, D., … Malison, R. T. (2006). A paradigm to investigate the regulation of cocaine self-administration in human cocaine users: a randomized trial. *Psychopharmacology*, *185*, 306–14.

Lyness, W. H., Friedle, N. M., & Moore, K. E. (1979). Destruction of dopaminergic nerve terminals in nucleus accumbens: effect on d-amphetamine self-administration. *Pharmacology, Biochemistry and Behavior*, *11*, 553–556.

Ma, S. T., Maier, E. Y., Ahrens, A. M., Schallert, T., & Duvauchelle, C. L. (2010). Repeated intravenous cocaine experience: development and escalation of pre-drug anticipatory 50-kHz ultrasonic vocalizations in rats. *Behavioural Brain Research*, *212*(1), 109–14. doi:10.1016/j.bbr.2010.04.001

MacLusky, N. J., & McEwen, B. S. (1978). Oestrogen modulates progestin receptor concentrations in some rat brain regions but not in others. *Nature*, *274*, 276–278.

Mahler, S. V, & Aston-Jones, G. S. (2012). Fos activation of selective afferents to ventral tegmental area during cue-induced reinstatement of cocaine seeking in rats. *The Journal of Neuroscience*, *32*(38), 13309–25. doi:10.1523/JNEUROSCI.2277-12.2012

Mani, S. K., & Portillo, W. (2010). Activation of progestin receptors in female reproductive behavior : Interactions with neurotransmitters. *Frontiers in Neuroendocrinology*, *31*(2), 157–171.

Mannella, P., & Brinton, R. D. (2006). Estrogen receptor protein interaction with phosphatidylinositol 3-kinase leads to activation of phosphorylated Akt and extracellular signal-regulated kinase 1/2 in the same population of cortical neurons: a unified mechanism of estrogen action. *The Journal of Neuroscience*, *26*(37), 9439–47. doi:10.1523/JNEUROSCI.1443-06.2006

Mannino, C. A., South, S. M., Inturrisi, C. E., & Quiñones-Jenab, V. (2005). Pharmacokinetics and effects of 17beta-estradiol and progesterone implants in ovariectomized rats. *The Journal of Pain*, *6*(12), 809–816. doi:10.1016/j.jpain.2005.07.007

Marco, N., Thirion, A., Mons, G., & Bougault, I. (1998). Activation of dopaminergic and cholinergic neurotransmission by tachykinin NK receptor stimulation : an in vivo microdialysis approach in guinea pig. *Neuropeptides*, *32*, 481–488.

Margolis, E. B., Lock, H., Hjelmstad, G. O., & Fields, H. L. (2006). The ventral tegmental area revisited: is there an electrophysiological marker for dopaminergic neurons? *The Journal of Physiology*, *577*, 907–24. doi:10.1113/jphysiol.2006.117069

Maroteaux, M., & Mameli, M. (2012). Cocaine evokes projection-specific synaptic plasticity of lateral habenula neurons. *Journal of Neuroscience*, *32*(36), 12641–12646. doi:10.1523/JNEUROSCI.2405-12.2012

Martínez, I., & Paredes, R. G. (2001). Only self-paced mating is rewarding in rats of both sexes. *Hormones and Behavior*, *40*(4), 510–7. doi:10.1006/hbeh.2001.1712

Martinez, L. a, Peterson, B. M., Meisel, R. L., & Mermelstein, P. G. (2014). Estradiol facilitation of cocaine-induced locomotor sensitization in female rats requires activation of mGluR5. *Behavioural Brain Research*, 50–53. doi:10.1016/j.bbr.2014.05.052

Matsuda, W., Furuta, T., Nakamura, K. C., Hioki, H., Fujiyama, F., Arai, R., & Kaneko, T. (2009). Single nigrostriatal dopaminergic neurons form widely spread and highly dense axonal arborizations in the neostriatum. *The Journal of Neuroscience*, *29*(2), 444–53. doi:10.1523/JNEUROSCI.4029-08.2009

Matsui, A., Jarvie, B. C., Robinson, B. G., Hentges, S. T., & Williams, J. T. (2014). Separate GABA afferents to dopamine neurons mediate acute action of opioids, development of tolerance, and expression of withdrawal. *Neuron*, *82*(6), 1346–56. doi:10.1016/j.neuron.2014.04.030

Matsumoto, J., Urakawa, S., Hori, E., Araujo, M. F. P. De, Sakuma, Y., & Ono, T. (2012). Neuronal responses in the nucleus accumbens shell during sexual behavior in male rats. *Journal of Neuroscience*, *32*(5), 1672–1686. doi:10.1523/JNEUROSCI.5140-11.2012

Matsumoto, R. R., McCracken, K. A., Pouw, B., Zhang, Y., & Bowen, W. D. (2002). Involvement of sigma receptors in the behavioral effects of cocaine: Evidence from novel ligands and antisense oligodeoxynucleotides. *Neuropharmacology*, *42*, 1043–1055. doi:10.1016/S0028-3908(02)00056-4

Mattson, B. J., & Morrell, J. I. (2005). Preference for cocaine- versus pup-associated cues differentially activates neurons expressing either Fos or cocaine- and amphetamine-regulated transcript in lactating, maternal rodents. *Neuroscience*, *135*(2), 315–28.

Mattson, B. J., Williams, S. E., Rosenblatt, J. S., & Morrell, J. I. (2003). Preferences for cocaine- or pup-associated chambers differentiates otherwise behaviorally identical postpartum maternal rats. *Psychopharmacology*, *167*(1), 1–8. doi:10.1007/s00213-002-1351-4

Mattson, B. J., Williams, S., Rosenblatt, J. S., & Morrell, J. I. (2001). Comparison of two positive reinforcing stimuli: Pups and cocaine throughout the postpartum period. *Behavioral Neuroscience*, *115*(3), 683–694. doi:10.1037//0735-7044.115.3.683

Matuszewich, L., Lorrain, D. S., & Hull, E. M. (2000). Dopamine release in the medial preoptic area of female rats in response to hormonal manipulation and sexual activity. *Behavioral Neuroscience*, *114*(4), 772–782. doi:10.1037//0735-7044.114.4.772

McArthur, S., McHale, E., & Gillies, G. E. (2007). The size and distribution of midbrain dopaminergic populations are permanently altered by perinatal glucocorticoid exposure in a sex- region- and time-specific manner. *Neuropsychopharmacology*, *32*(7), 1462–76. doi:10.1038/sj.npp.1301277

McCance-Katz, E. F., Carroll, K. M., & Rounsaville, B. J. (1999). Gender differences in treatment-seeking cocaine abusers — Implications for treatment and prognosis. *The American Journal on Addictions*, *8*, 300–311.

McCarthy, M. M., Kaufman, L. C., Brooks, P. J., Pfaff, D. W., & Schwartz-Giblin, S. (1995). Estrogen modulation of mRNA levels for the two forms of glutamic acid decarboxylase (GAD) in female rat brain. *The Journal of Comparative Neurology*, *360*(4), 685–97. doi:10.1002/cne.903600412

McEwen, B. S., & Alves, S. E. (1999). Estrogen actions in the central nervous system. *Endocrine Reviews*, *20*(3), 279–307. doi:10.1210/edrv.20.3.0365

McFarland, K., & Kalivas, P. W. (2001). The circuitry mediating cocaine-induced reinstatement of drug- seeking behavior. *Journal of Neuroscience*, *21*(21), 8655–8663.

Meisel, R. L., Camp, D. M., & Robinson, T. E. (1993). A microdialysis study of ventral striatal dopamine during sexual behavior in female Syrian hamsters. *Behavioural Brain Research*, *55*, 151–157.

Mello, N. K., Bowen, C. A., & Mendelson, J. H. (2002). Comparison of plasma cocaine levels during a "binge" pattern of cocaine administration in male and female rhesus monkeys. *Psychopharmacology*, *164*(1), 19–26. doi:10.1007/s00213-002-1188-x

Mello, N. K., Knudson, I. M., & Mendelson, J. H. (2007). Sex and menstrual cycle effects on progressive ratio measures of cocaine self-administration in cynomolgus monkeys. *Neuropsychopharmacology*, *32*, 1956–1966. doi:10.1038/sj.npp.1301314

Mello, N. K., Mendelson, J. H., Kelly, M., & Bowen, C. A. (2000). The effects of cocaine on basal and human chorionic gonadotropin-stimulated ovarian steroid hormones in female rhesus monkeys. *The Journal of Pharmacology and Experimental Therapeutics*, *294*(3), 1137–1145.

Mello, N. K., Sarnyai, Z., Mendelson, J. H., Drieze, J. M., & Kelly, M. (1993). Acute effects of cocaine on anterior pituitary hormones in male and female rhesus monkeys. *The Journal of Pharmacology and Experimental Therapeutics*, *266*(2), 804–811.

Mendelson, J. H., Mello, N. K., Sholar, M. B., Siegel, A. J., Kaufman, M. J., Levin, J. M., … Cohen, B. M. (1999). Cocaine pharmacokinetics in men and in women during the follicular and luteal phases of the menstrual cycle. *Neuropsychopharmacology*, *21*, 294–303. doi:10.1016/S0893-133X(99)00020-2

Merchenthaler, I., Lane, M. V, Numan, S., & Dellovade, T. L. (2004). Distribution of estrogen receptor alpha and beta in the mouse central nervous system: in vivo autoradiographic and immunocytochemical analyses. *The Journal of Comparative Neurology*, *473*(2), 270–91. doi:10.1002/cne.20128

Mermelstein, P. G., & Becker, J. B. (1995). Increased extracellular dopamine in the nucleus accumbens and striatum of the female rat during paced copulatory behavior. *Behavioral Neuroscience*, *109*(2), 354–65.

Micevych, P., & Dominguez, R. (2009). Membrane estradiol signaling in the brain. *Frontiers in Neuroendocrinology*, *30*(3), 315–27. doi:10.1016/j.yfrne.2009.04.011

Mihm, M., Gangooly, S., & Muttukrishna, S. (2011). The normal menstrual cycle in women. *Animal Reproduction Science*, *124*(3-4), 229–36. doi:10.1016/j.anireprosci.2010.08.030

Mikhail, G. (1967). Sex steroids in blood. *Clinical Obstetrics and Gynecology*, *10*(1), 29–39.

Miller, S. M., & Lonstein, J. S. (2005). Dopamine D1 and D2 receptor antagonism in the preoptic area produces different effects on maternal behavior in lactating rats. *Behavioral Neuroscience*, *119*(4), 1072–1083.

Miller, S. M., & Lonstein, J. S. (2009). Dopaminergic projections to the medial preoptic area of postpartum rats. *Neuroscience*, *159*(4), 1384–96.

Missale, C., Nash, S. R., Robinson, S. W., Jaber, M., & Caron, M. G. (1998). Dopamine receptors : From structure to function. *Physiological Reviews*, *78*(1), 189–226.

Mitra, S. W., Hoskin, E., Yudkovitz, J., Pear, L., Wilkinson, H. A., Hayashi, S., … Alves, S. E. (2003). Immunolocalization of estrogen receptor beta in the mouse brain : Comparison with estrogen receptor alpha. *Endocrinology*, *144*(5), 2055–2067. doi:10.1210/en.2002-221069

Morgan, H. D., Watchus, J. a, Milgram, N. W., & Fleming, A. S. (1999). The long lasting effects of electrical simulation of the medial preoptic area and medial amygdala on maternal behavior in female rats. *Behavioural Brain Research*, *99*(1), 61–73. doi:10.1016/S0166-4328(98)00070-9

Moses, J., Loucks, J. a, Watson, H. L., Matuszewich, L., & Hull, E. M. (1995). Dopaminergic drugs in the medial preoptic area and nucleus accumbens: effects on motor activity, sexual motivation, and sexual performance. *Pharmacology, Biochemistry, and Behavior*, *51*(4), 681–6.

Nair-Roberts, R. G., Chatelain-Badie, S. D., Benson, E., White-Cooper, H., Bolam, J. P., & Ungless, M. A. (2008). Stereological estimates of dopaminergic, GABAergic and glutamatergic neurons in the ventral tegmental area, substantia nigra and retrorubral field in the rat. *Neuroscience*, *152*(4), 1024–31. doi:10.1016/j.neuroscience.2008.01.046

Nephew, B. C., & Febo, M. (2012). Effects of cocaine on maternal behavior and neurochemistry. *Current Neuropharmacology*, *10*(1), 53–63. doi:10.2174/157015912799362760

Nestler, E. J. (2005). The neurobiology of cocaine addiction. *Science & Practice Perspectives*, *3*(1), 4–10.

Nicola, S. M., Yun, I. A., Wakabayashi, K. T., & Fields, H. L. (2004). Firing of nucleus accumbens neurons during the consummatory phase of a discriminative stimulus task depends on previous reward predictive cues. *Journal of Neurophysiology*, *91*(4), 1866–82. doi:10.1152/jn.00658.2003

Nieh, E. H., Kim, S.-Y., Namburi, P., & Tye, K. M. (2013). Optogenetic dissection of neural circuits underlying emotional valence and motivated behaviors. *Brain Research*, *1511*, 73–92. doi:10.1016/j.brainres.2012.11.001

Niyomchai, T., Akhavan, A., Festa, E. D., Lin, S.-N., Lamm, L., Foltz, R., & Quiñones-Jenab, V. (2006). Estrogen and progesterone affect cocaine pharmacokinetics in female rats. *Brain Research Bulletin*, *68*(5), 310–4. doi:10.1016/j.brainresbull.2005.09.001

Niyomchai, T., Jenab, S., Festa, E. D., Akhavan, A., & Quiñones-Jenab, V. (2006). Effects of short- and long-term estrogen and progesterone replacement on behavioral responses and c-fos mRNA levels in female rats after acute cocaine administration. *Brain Research*, *1126*(1), 193–9. doi:10.1016/j.brainres.2006.07.099

Niyomchai, T., Russo, S. J., Festa, E. D., Akhavan, A., Jenab, S., & Quiñones-Jenab, V. (2005). Progesterone inhibits behavioral responses and estrogen increases corticosterone levels after acute cocaine administration. *Pharmacology, Biochemistry, and Behavior*, *80*(4), 603–10.

Numan, M., & Numan, M. J. (2003). Projection sites of medial preoptic area and ventral bed nucleus of the stria terminalis neurons that express fos during maternal behavior in female rats. *Journal of Neuroendocrinology*, *9*(5), 369–384. doi:10.1046/j.1365-2826.1997.t01-1-00597.x

Numan, M., Numan, M. J., Pliakou, N., Stolzenberg, D. S., Mullins, O. J., Murphy, J. M., & Smith, C. D. (2005). The effects of D1 or D2 dopamine receptor antagonism in the medial preoptic area, ventral pallidum, or nucleus accumbens on the maternal retrieval response and other aspects of maternal behavior in rats. *Behavioral Neuroscience*, *119*(6), 1588–604. doi:10.1037/0735-7044.119.6.1588

Numan, M., Numan, M. J., Schwarz, J. M., Neuner, C. M., Flood, T. F., & Smith, C. D. (2005). Medial preoptic area interactions with the nucleus accumbens-ventral pallidum circuit and maternal behavior in rats. *Behavioral Brain Research*, *158*(1), 53–68. doi:10.1016/j.bbr.2004.08.008

Numan, M., Rosenblatt, J. S., & Komisaruk, B. R. (1977). Medial preoptic area and onset of maternal behavior in the rat. *Journal Of Comparative and Physiological Psychology*, *91*(1), 146–164.

Numan, M., & Smith, H. G. (1984). Maternal behavior in rats: evidence for the involvement of preoptic projections to the ventral tegmental area. *Behavioral Neuroscience*, *98*(4), 712–27.

Numan, M., Stolzenberg, D. S., Dellevigne, A. A., Correnti, C. M., & Numan, M. J. (2009). Temporary inactivation of ventral tegmental area neurons with either muscimol or baclofen reversibly disrupts maternal behavior in rats through different underlying mechanisms. *Behavioral Neuroscience*, *123*(4), 740–751.

O'Connell, L. A., & Hofmann, H. A. (2011). The vertebrate mesolimbic reward system and social behavior network: A comparative synthesis. *The Journal of Comparative Neurology*, *519*(18), 3599–639. doi:10.1002/cne.22735

O'Donnell, P., & Grace, A. (1995). Synaptic interactions accumbens neurons : input among excitatory afferents to nucleus hippocampal gating of prefrontal cortical. *The Journal of Neuroscience*, *15*(5), 3622–3639.

Olsen, C. M. (2011). Natural rewards , neuroplasticity , and non-drug addictions. *Neuropharmacology*, *61*(7), 1109–1122. doi:10.1016/j.neuropharm.2011.03.010

Österlund, M. (1998). Differential distribution and regulation of estrogen receptor-α and -β mRNA within the female rat brain. *Molecular Brain Research*, *54*(1), 175–180.

Pang, Y., Dong, J., & Thomas, P. (2013). Characterization, neurosteroid binding and brain distribution of human membrane progesterone receptors δ and {epsilon} (mPRδ and mPR{epsilon}) and mPRδ involvement in neurosteroid inhibition of apoptosis. *Endocrinology*, *154*(1), 283–95. doi:10.1210/en.2012-1772

Panzica, G. C., Balthazart, J., Frye, C. A., Garcia-Segura, L. M., Herbison, A. E., Mensah-Nyagan, A. G., … Melcangi, R. C. (2011). Milestones on steroids and the nervous system : 10 years of basic and neuroendocrinology. *Journal of Neuroendocrinology*, *24*(1), 1–15. doi:10.1111/j.1365-2826.2011.02265.x

Parada, M., Chamas, L., Censi, S., Coria-Avila, G., & Pfaus, J. G. (2010). Clitoral stimulation induces conditioned place preference and Fos activation in the rat. *Hormones and Behavior*, *57*(2), 112–8. doi:10.1016/j.yhbeh.2009.05.008

Paredes, R. G., Tzschentke, T., & Nakach, N. (1998). Lesions of the medial preoptic area/anterior hypothalamus (MPOA/AH) modify partner preference in male rats. *Brain Research*, *813*(1), 1–8.

Paredes, R. G., & Vazquez, B. (1999). What do female rats like about sex? Paced mating. *Behavioural Brain Research*, *105*(1), 117–127. doi:10.1016/S0166-4328(99)00087-X

Parsons, B., Rainbow, T. C., MacLusky, N. J., & McEwen, B. S. (1982). Progestin receptor levels in rat hypothalamic and limbic nuclei. *The Journal of Neuroscience*, *2*(10), 1446–1452.

Pasqualini, C., Olivier, V., Guibert, B., Frain, O., & Leviel, V. (2002). Acute stimulatory effect of estradiol on striatal dopamine synthesis. *Journal of Neurochemistry*, *65*(4), 1651–1657. doi:10.1046/j.1471-4159.1995.65041651.x

Patterson, M., Murphy, K. G., Thompson, E. L., Smith, K. L., Meeran, K., Ghatei, M. a, & Bloom, S. R. (2006). Microinjection of galanin-like peptide into the medial preoptic area stimulates food intake in adult male rats. *Journal of Neuroendocrinology*, *18*(10), 742–7. doi:10.1111/j.1365-2826.2006.01473.x

Paxinos, G., & Watson, C. (2007). The rat brain in stereotaxic coordinates. New York: Academic Press.

Peluso, J. J., Liu, X., & Romak, J. (2007). Progesterone maintains basal intracellular adenosine triphosphate levels and viability of spontaneously immortalized granulosa cells by promoting an interaction between 14-3-3sigma and ATP synthase beta/precursor through a protein kinase G-dependent mecha. *Endocrinology*, *148*(5), 2037–44. doi:10.1210/en.2006-1603

Peluso, J. J., & Pappalardo, A. (2004). Progesterone regulates granulosa cell viability through a protein kinase G-dependent mechanism that may involve 14-3-3sigma. *Biology of Reproduction*, *71*(6), 1870–8. doi:10.1095/biolreprod.104.031716

Peoples, L. L., Gee, F., Bibi, R., & West, M. O. (1998). Phasic firing time locked to cocaine self-infusion and locomotion : dissociable riring patterns of single nucleus accumbens neurons in the rat. *The Journal of Neuroscience*, *18*(18), 7588–7598.

Peoples, L. L., & West, M. O. (1996). Phasic firing of single neurons in the rat nucleus accumbens correlated with the timing of intravenous cocaine self-administration. *The Journal of Neuroscience*, *16*(10), 3459–3473.

Pereira, M., & Morrell, J. I. (2010). The medial preoptic area is necessary for motivated choice of pup- over cocaine-associated environments by early postpartum rats. *Neuroscience*, *167*(2), 216–31.

Pereira, M., & Morrell, J. I. (2011). Functional mapping of the neural circuitry of rat maternal motivation: effects of site-specific transient neural inactivation. *Journal of Neuroendocrinology*, *23*(11), 1020–35. doi:10.1111/j.1365-2826.2011.02200.x

Peris, J., Decambre, N., Coleman-Hardee, M. L., & Simpkins, J. W. (1991). Estradiol enhances behavioral sensitization to cocaine and amphetamine-stimulated striatal [3H]dopamine release. *Brain Research*, *566*(1-2), 255–64.

Perry, A. N., Westenbroek, C., & Becker, J. B. (2013). Impact of pubertal and adult estradiol treatments on cocaine self-administration. *Hormones and Behavior*, *64*(4), 573–8. doi:10.1016/j.yhbeh.2013.08.007

Perry, J. L., Nelson, S. E., & Carroll, M. E. (2008). Impulsive choice as a predictor of acquisition of IV cocaine self- administration and reinstatement of cocaine-seeking behavior in male and female rats. *Experimental and Clinical Psychopharmacology*, *16*, 165–177. doi:10.1037/1064-1297.16.2.165

Petersen, S. L., Intlekofer, K. A., Moura-Conlon, P. J., Brewer, D. N., Del Pino Sans, J., & Lopez, J. A. (2013). Non-classical progesterone signalling molecules in the nervous system. *Journal of Neuroendocrinology*, (18), 991–1001. doi:10.1111/jne.12060

Pettit, H. O., Ettenberg, A., Bloom, F. E., & Koob, G. F. (1984). Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. *Psychopharmacology*, *84*(2), 167–173. doi:10.1007/BF00427441

Pfaus, J. G., & Heeb, M. M. (1997). Implications of immediate-early gene induction in the brain following sexual stimulation of female and male rodents. *Brain Research Bulletin*, *44*(4), 397–407.

Pfaus, J. G., Marcangione, C., Smith, W. J., Manitt, C., & Abillamaa, H. (1996). Differential induction of Fos in the female rat brain following different amounts of vaginocervical stimulation: modulation by steroid hormones. *Brain Research*, *741*(1-2), 314–30.

Pfaus, J. G., & Phillips, A. G. (1991). Role of dopamine in anticipatory and consummatory aspects of sexual behavior in the male rat. *Behavioral Neuroscience*, *105*(5), 727–743.

Pfaus, J. G., & Scepkowski, L. A. (2005). The biologic basis for libido. *Current Sexual Health Reports*, *2*(4), 95–100. doi:10.1007/s11930-005-0010-2

Pfaus, J. G., Wilkins, M. F., Dipietro, N., Benibgui, M., Toledano, R., Rowe, A., & Couch, M. C. (2010). Inhibitory and disinhibitory effects of psychomotor stimulants and depressants on the sexual behavior of male and female rats. *Hormones and Behavior*, *58*(1), 163–76. doi:10.1016/j.yhbeh.2009.10.004

Phillips, G. D., Robbins, T. W., & Everitt, B. J. (1994). Bilateral intra-accumbens self-administration of d-amphetamine : Antagonism with intra-accumbens SCH-23390 and sulpiride. *Psychopharmacology*, *114*, 477–485.

Phillipson, O. T., & Griffiths, A. C. (1985). The topographic order of inputs to nucleus accumbens in the rat. *Neuroscience*, *16*(2), 275–296. doi:10.1016/0306-4522(85)90002-8

Pierce, R. C., & Kumaresan, V. (2006). The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? *Neuroscience and Biobehavioral Reviews*, *30*(2), 215–38. doi:10.1016/j.neubiorev.2005.04.016

Potter, D. A., Futher, M. F., Eddy, C. A., Siler-Khodr, T. M., King, T. S., & Schenken, R. S. (1999). Low-dose follicular-phase cocaine administration disrupts menstrual and ovarian cyclicity in rhesus monkeys. *Journal of the Society for Gynecologic Investigation*, *6*, 88. doi:10.1177/107155769900600207

Powers, B., & Valenstein, E. S. (1972). Sexual receptivity: Facilitation by medial preoptic lesions in female rats. *Science*, *175*(4025), 1003–1005. doi:10.1126/science.175.4025.1003

Powers, J. B., Newman, S. W., & Bergondy, M. L. (1987). MPOA and BNST lesions in male Syrian hamsters: differential effects on copulatory and chemoinvestigatory behaviors. *Behavioral Brain Research*, *23*(3), 181–95.

Prossnitz, E. R., & Maggiolini, M. (2009). Mechanisms of estrogen signaling and gene expression via GPR30. *Molecular and Cellular Endocrinology*, *308*(1-2), 32–8. doi:10.1016/j.mce.2009.03.026

Quadros, P. S. (2002). Sex differences in progesterone receptor expression: A potential mechanism for estradiol-mediated sexual differentiation. *Endocrinology*, *143*(10), 3727–3739. doi:10.1210/en.2002-211438

Quadros, P. S., Pfau, J. L., & Wagner, C. K. (2007). Distribution of progesterone receptor immunoreactivity in the fetal and neonatal rat forebrain. *Comparative and General Pharmacology*, *56*(December), 42–56.

Quiñones-Jenab, V. (2006). Why are women from Venus and men from Mars when they abuse cocaine? *Brain Research*, *1126*(1), 200–3. doi:10.1016/j.brainres.2006.08.109

Quiñones-Jenab, V., & Jenab, S. (2010). Progesterone attenuates cocaine-induced responses. *Hormones and Behavior*, *58*(1), 22–32.

Raisman, G., & Field, P. M. (1971). Sexual dimorphism in the preoptic area of the rat. *Science*, *173*(3998), 731–733.

Ramirez, V. D., Zheng, J., & Siddique, K. M. (1996). Membrane receptors for estrogen, progesterone, and testosterone in the rat brain: Fantasy or reality. *Cellular and Molecular Neurobiology*, *16*(2), 175–198. doi:10.1007/BF02088175

Ramôa, C. P., Doyle, S. E., Naim, D. W., & Lynch, W. J. (2013). Estradiol as a mechanism for sex differences in the development of an addicted phenotype following extended access cocaine self-administration. *Neuropsychopharmacology*, *38*, 1698–705. doi:10.1038/npp.2013.68

Ranaldi, R., Kest, K., Zellner, M. R., Lubelski, D., Muller, J., Cruz, Y., & Saliba, M. (2011). The effects of VTA NMDA receptor antagonism on reward-related learning and associated c-fos expression in forebrain. *Behavioural Brain Research*, *216*(1), 424–32. doi:10.1016/j.bbr.2010.08.026

Reed, S. C., Evans, S. M., Bedi, G., Rubin, E., & Foltin, R. W. (2011). The effects of oral micronized progesterone on smoked cocaine self-administration in women. *Hormones and Behavior*, *59*(2), 227–35. doi:10.1016/j.yhbeh.2010.12.009

Reith, M. E. A., Li, M.-Y., & Yan, Q.-S. (1997). Extracellular dopamine, norepinephrine, and serotonin in the ventral tegmental area and nucleus accumbens of freely moving rats during intracerebral dialysis following systemic administration of cocaine and other uptake blockers. *Psychopharmacology*, *134*(3), 309–317. doi:10.1007/s002130050454

Ribeiro, A. C., Musatov, S., Shteyler, A., Simanduyev, S., Arrieta-Cruz, I., Ogawa, S., & Pfaff, D. W. (2012). siRNA silencing of estrogen receptor-α expression specifically

in medial preoptic area neurons abolishes maternal care in female mice. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(40), 16324–9. doi:10.1073/pnas.1214094109

Ritz, M. C., Lamb, R. J., Goldberg, S. R., & Kuhar, M. J. (1987). Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science*, *237*(4819), 1219–1223.

Rivas, F. J., & Mir, D. (1990). Effects of nucleus accumbens lesion on female rat sexual receptivity and proceptivity in a partner preference paradigm. *Behavioural Brain Research*, *41*(3), 239–249. doi:10.1016/0166-4328(90)90111-Q

Rivas, F. J., & Mir, D. (1991). Accumbens lesion in female rats increases mount rejection without modifying lordosis. *Revista Española de Fisiología*, *47*(1), 1–6.

Robbins, S. J., Ehrman, R. N., Childress, A. R., & O'Brien, C. P. (1999). Comparing levels of cocaine cue reactivity in male and female outpatients. *Drug and Alcohol Dependence*, *53*(3), 223–30.

Roberts, D. C., Bennett, S. A., & Vickers, G. J. (1989). The estrous cycle affects cocaine self-administration on a progressive ratio schedule in rats. *Psychopharmacology*, *102*(7-8), 314–20.

Roberts, D. C., & Koob. (1982). Disruption of cocaine self-administration following 6-hydroxydopamine lesions of the ventral tegmental area in rats. *Pharmacology Biochemistry and Behavior*, *17*, 901–904.

Roberts, D. C., Koob, G. F., Klonoff, P., & Fibiger, H. C. (1980). Extinction and recovery of cocaine self-administration following 6-hydroxydopamine lesions of the nucleus accumbens. *Pharmacology, Biochemistry, and Behavior*, *12*(5), 781–7.

Robinson, T. E., & Becker, J. B. (1982). Behavioral sensitization is accompanied by an enhancement in amphetamine-stimulated dopamine release from sriatal tissue in vitro. *European Journal of Pharmacology*, *85*, 253–254.

Robinson, T. E., Becker, J. B., & Presty, S. K. (1982). Long-term facilitation of amphetamine-induced rotational behavior and striatal dopamine release produced by a single exposure to amphetamine: sex differences. *Brain Research*, *253*(1-2), 231–41.

Robinson, T. E., & Berridge, K. C. (1993). The neural basis of drug craving: an incentive-sensitization theory of addiction. *Brain Research Reviews*, *8*, 247–291.

Robinson, T. E., & Berridge, K. C. (2008). The incentive sensitization theory of addiction: some current issues. *Philosophical Transactions of the Royal Society B*, *363*(1507), 3137–46. doi:10.1098/rstb.2008.0093

Rodd-Henricks, Z. A., McKinzie, D. L., Li, T.-K., Murphy, J. A., & McBride, W. J. (2002). Cocaine is self-administered into the shell but not the core of the nucleus accumbens of Wistar rats. *The Journal of Pharmacology and Experimental Therapeutics*, *303*(3), 1216–1226. doi:10.1124/jpet.102.038950.blocked

Rodriguez-Sierra, J. F., & Terasawa, E. (1979). Lesions of the preoptic area facilitate lordosis behavior in male and female guinea pigs. *Brain Research Bulletin*, *4*(4), 513–7.

Roepke, T. A., Qiu, J., Bosch, M. A., Rønnekleiv, O. K., & Kelly, M. J. (2009). Cross-talk between membrane-initiated and nuclear-initiated oestrogen signalling in the hypothalamus. *Journal of Neuroendocrinology*, *21*(4), 263–70. doi:10.1111/j.1365-2826.2009.01846.x

Rose, J. D. (1990). Tectoreticular neuronal activity associated with behavior and progesterone action during the induction of lordosis responding in hamsters. *Abstr. Soc. Neurosci.*

Roth, M. E., & Carroll, M. E. (2004). Sex differences in the escalation of intravenous cocaine intake following long- or short-access to cocaine self-administration. *Pharmacology, Biochemistry and Behavior*, *78*(2), 199–207. doi:10.1016/j.pbb.2004.03.018

Rothman, R. B., Baumann, M. H., Dersch, C. M., Romero, D. V, Rice, K. C., Carroll, F. I. V. Y., & Partilla, J. S. (2001). Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. *Synapse*, *41*, 32–41.

Russo, S. J., Festa, E. D., Fabian, S. J., Gazi, F. M., Kraish, M., Jenab, S., & Quin, V. (2003). Gonadal hormones differentially modulate cocaine-induced conditioned place preference in male and female rats. *Neuroscience*, *120*, 523–533.

Russo, S. J., Jenab, S., Fabian, S. J., Festa, E. D., Kemen, L. M., & Quiñones-Jenab, V. (2003). Sex differences in the conditioned rewarding effects of cocaine. *Brain Research*, *970*(1-2), 214–20.

Saddoris, M. P., Sugam, J. A., Cacciapaglia, F., & Carelli, R. M. (2014). Rapid dopamine dynamics in the accumbens core and shell: Learning and action. *Frontiers in BioScience*, *5*, 273–288.

Sakaki, M., & Mather, M. (2012). How reward and emotional stimuli induce different reactions across the menstrual cycle. *Social and Personality Psychology Compass*, *6*(1), 1–17. doi:10.1111/j.1751-9004.2011.00415.x

Salamone, J. D., Cousins, M. S., & Snyder, B. J. (1997). Behavioral functions of nucleus accumbens dopamine : empirical and conceptual problems with the anhedonia hypothesis. *Neuroscience and Biobehavioral Reviews*, *21*(3), 341–359.

Santollo, J., Torregrossa, A.-M., & Eckel, L. A. (2011). Estradiol acts in the medial preoptic area, arcuate nucleus, and dorsal raphe nucleus to reduce food intake in ovariectomized rats. *Hormones and Behavior*, *60*(1), 86–93.

Schensul, J. J., Convey, M., & Burkholder, G. (2005). Challenges in measuring concurrency, agency and intentionality in polydrug research. *Addictive Behaviors*, *30*(3), 571–4. doi:10.1016/j.addbeh.2004.05.022

Schultz, K. N., von Esenwein, S. A., Hu, M., Bennett, A. L., Kennedy, R. T., Musatov, S., … Becker, J. B. (2009). Viral vector-mediated overexpression of estrogen receptor-alpha in striatum enhances the estradiol-induced motor activity in female rats and estradiol-modulated GABA release. *The Journal of Neuroscience*, *29*(6), 1897–903. doi:10.1523/JNEUROSCI.4647-08.2009

Schultz, W. (1998). Predictive Reward Signal of Dopamine Neurons. *Journal of Neurophysiology*, *80*, 1–27.

Schultz, W. (2000). Multiple reward signals in the brain. *Nature Reviews Neuroscience*, *1*(December), 199–207.

Schultz, W. (2002). Getting Formal with Dopamine and Reward. *Neuron*, *36*(2), 241–263. doi:10.1016/S0896-6273(02)00967-4

Seecof, R., & Tennant, F. S. (1986). Subjective perceptions to the intravenous “rush” of heroin and cocaine in opioid addicts. *The American Journal of Drug and Alcohol Abuse*, *12*(1-2), 79–87.

Segal, D. S., & Mandell, A. J. (1974). Long-term administration of d-amphetamine: Progressive augmentation of motor activity and stereotypy. *Pharmacology Biochemistry and Behavior*, *2*(2), 249–255. doi:10.1016/0091-3057(74)90060-4

Segarra, A. C., Agosto-Rivera, J. L., Febo, M., Lugo-Escobar, N., Menéndez-Delmestre, R., Puig-Ramos, A., & Torres-Diaz, Y. M. (2010). Estradiol: a key biological substrate mediating the response to cocaine in female rats. *Hormones and Behavior*, *58*(1), 33–43. doi:10.1016/j.yhbeh.2009.12.003

Segarra, A. C., Torres-Díaz, Y. M., Silva, R. D., Puig-Ramos, A., Menéndez-Delmestre, R., Rivera-Bermúdez, J. G., … Agosto-Rivera, J. L. (2014). Estrogen receptors mediate estradiol's effect on sensitization and CPP to cocaine in female rats: Role of contextual cues. *Hormones and Behavior*, *65*(2), 77–87. doi:10.1016/j.yhbeh.2013.12.007

Seip, K. M., & Morrell, J. I. (2007). Increasing the incentive salience of cocaine challenges preference for pup- over cocaine-associated stimuli during early postpartum: place preference and locomotor analyses in the lactating female rat. *Psychopharmacology*, *194*(3), 309–19. doi:10.1007/s00213-007-0841-9

Seip, K. M., & Morrell, J. I. (2009). Transient inactivation of the ventral tegmental area selectively disrupts the expression of conditioned place preference for pup- but not cocaine-paired contexts. *Behavioral Neuroscience*, *123*(6), 1325–38.

Seip, K. M., Pereira, M., Wansaw, M. P., Reiss, J. I., Dziopa, E. I., & Morrell, J. I. (2008). Incentive salience of cocaine across the postpartum period of the female rat. *Psychopharmacology*, *199*(1), 119–30. doi:10.1007/s00213-008-1140-9

Sell, S. L., Scalzitti, J. M., Thomas, M. L., & Cunningham, K. A. (2000). Influence of ovarian hormones and estrous cycle on the behavioral response to cocaine in female rats. *The Journal of Pharmacology and Experimental Therapeutics*, *293*(3), 879–86.

Sellings, L. H. L., McQuade, L. E., & Clarke, P. B. S. (2006). Characterization of dopamine-dependent rewarding and locomotor stimulant effects of intravenously-administered methylphenidate in rats. *Neuroscience*, *141*(3), 1457–68. doi:10.1016/j.neuroscience.2006.04.040

Shank, E. J., Seitz, P. K., Bubar, M. J., Stutz, S. J., & Cunningham, K. A. (2007). Selective ablation of GABA neurons in the ventral tegmental area increases spontaneous locomotor activity. *Behavioral Neuroscience*, *121*(6), 1224–1233.

Shughrue, P. (1998). Comparative distribution of estrogen receptor-α (ER-α) and β (ER-β) mRNA in the rat pituitary, gonad, and reproductive tract. *Steroids*, *63*(10), 498–504.

Shughrue, P. J., Bushnell, C. D., & Dorsa, D. M. (1992). Estrogen receptor messenger ribonucleic acid in female rat brain during the estrous cycle: a comparison with ovariectomized females and intact males. *Endocrinology*, *131*(1), 381–388.

Silverthorn, D. U. (2009). *Human Physiology : An Integrated Approach* (5th Editio., p. 998). San Francisco: Pearson/Benjamin Cummings.

Simerly, R. B., Chang, C., Muramatsu, M., & Swanson, L. W. (1990). Distribution of androgen and estrogen receptor mRNA-containing cells in the rat brain: an in situ hybridization study. *The Journal of Comparative Neurology*, *294*(1), 76–95. doi:10.1002/cne.902940107

Simerly, R. B., & Swanson, L. W. (1986). The organization of neural inputs to the medial preoptic nucleus of the rat. *The Journal of Comparative Neurology*, *246*(3), 312–42. doi:10.1002/cne.902460304

Simerly, R. B., & Swanson, L. W. (1988). Projections of the medial preoptic nucleus: a Phaseolus vulgaris leucoagglutinin anterograde tract-tracing study in the rat. *The Journal of Comparative Neurology*, *270*(2), 209–42. doi:10.1002/cne.902700205

Simmons, D. A., Hoffman, N. W., & Yahr, P. (2011). A forebrain-retrorubral pathway involved in male sex behavior is GABAergic and activated with mating in gerbils. *Neuroscience*, *175*, 162–8. doi:10.1016/j.neuroscience.2010.11.048

Simmons, D. A., & Yahr, P. (2003). GABA and glutamate in mating-activated cells in the preoptic area and medial amygdala of male gerbils. *The Journal of Comparative Neurology*, *459*(3), 290–300.

Singha, A. K., Mccance-Katz, E. F., Petrakis, I., Kosten, T. R., & Oliveto, A. (2000). Sex differences in self-reported and physiological response to oral cocaine and placebo in humans. *American Journal of Drug and Alcohol Abuse*, *26*(4), 643–657.

Sinha, R., Fox, H., Hong, K.-I., Sofuoglu, M., Morgan, P. T., & Bergquist, K. T. (2007). Sex steroid hormones, stress response, and drug craving in cocaine-dependent women: implications for relapse susceptibility. *Experimental and Clinical Psychopharmacology*, *15*(5), 445–52.

Siqueland, L., Crits-christoph, P., Barber, J. P., Griffin, M. L., Thase, M. E., Daley, D., … Gladis, M. (2002). Retention in psychosocial treatment of cocaine dependence : Predictors and impact on outcome. *The American Journal on Addictions*, *11*, 24–40.

Smith, K. S., Tindell, A. J., Aldridge, J. W., & Berridge, K. C. (2010). Ventral pallidum roles in reward and motivation. *Behavioral Brain Research*, *196*(2), 155–167.

Sofuoglu, M., Babb, D. A., & Hatsukami, D. K. (2001). Progesterone treatment during the early follicular phase of the menstrual cycle: effects on smoking behavior in women. *Pharmacology, Biochemistry and Behavior*, *69*(1-2), 299–304.

Sofuoglu, M., Babb, D. A., & Hatsukami, D. K. (2002). Effects of progesterone treatment on smoked cocaine response in women. *Pharmacology, Biochemistry, and Behavior*, *72*(1-2), 431–5.

Sofuoglu, M., Dudish-Poulsen, S., Nelson, D., Pentel, P. R., & Hatsukami, D. K. (1999). Sex and menstrual cycle differences in the subjective effects from smoked cocaine in humans. *Experimental and Clinical Psychopharmacology*, *7*(3), 274–83.

Sofuoglu, M., Mitchell, E., & Kosten, T. R. (2004). Effects of progesterone treatment on cocaine responses in male and female cocaine users. *Pharmacology, Biochemistry, and Behavior*, *78*(4), 699–705. doi:10.1016/j.pbb.2004.05.004

Sokolowski, J. ., Conlan, a. ., & Salamone, J. . (1998). A microdialysis study of nucleus accumbens core and shell dopamine during operant responding in the rat. *Neuroscience*, *86*(3), 1001–1009. doi:10.1016/S0306-4522(98)00066-9

Spanagel, R., & Weiss, F. (1999). The dopamine hypothesis of reward: past and current status. *Trends in Neurosciences*, *22*(11), 521–7.

Spiteri, T., Ogawa, S., Musatov, S., Pfaff, D. W., & Ågmo, A. (2012). The role of the estrogen receptor α in the medial preoptic area in sexual incentive motivation, proceptivity and receptivity, anxiety, and wheel running in female rats. *Behavioural Brain Research*, *230*(1), 11–20.

Spratt, D. P., & Herbison, A. E. (1997). Regulation of preoptic area gonadotrophin-releasing hormone (GnRH) mRNA expression by gonadal steroids in the long-term gonadectomized male rat. *Molecular Brain Research*, *47*(1-2), 125–33.

Stack, E. C., Balakrishnan, R., Numan, M. J., & Numan, M. (2002). A functional neuroanatomical investigation of the role of the medial preoptic area in neural circuits regulating maternal behavior. *Behavioural Brain Research*, *131*(1-2), 17–36.

Stefanik, M. T., Kupchik, Y. M., Brown, R. M., & Kalivas, P. W. (2013). Optogenetic evidence that pallidal projections, not nigral projections, from the nucleus accumbens core are necessary for reinstating cocaine seeking. *The Journal of Neuroscience*, *33*(34), 13654–62. doi:10.1523/JNEUROSCI.1570-13.2013

Stefanik, M. T., Moussawi, K., Kupchik, Y. M., Smith, K. C., Miller, R. L., Huff, M. L., … LaLumiere, R. T. (2013). Optogenetic inhibition of cocaine seeking in rats. *Addiction Biology*, *18*(1), 50–3. doi:10.1111/j.1369-1600.2012.00479.x

Stein, L. (1968). Chemistry of Reward and Punishment. In D. H. Efron (Ed.), *Psychopharmacology: A Review of Progress, 1957–1967* (p. 105). Washington D.C.: U.S. Government Printing Office.

Stobbs, S. H., Ohran, A. J., Lassen, M. B., Allison, D. W., Brown, J. E., & Steffensen, S. C. (2004). Ethanol suppression of ventral tegmental area GABA neuron electrical transmission involves N -Methyl- D -aspartate receptors. *The Journal of Pharmacology and Experimental Therapeutics*, *311*, 282–289. doi:10.1124/jpet.104.071860.The

Stolzenberg, D. S., & Numan, M. (2010). Hypothalamic interaction with the mesolimbic DA system in the control of the maternal and sexual behaviors in rats. *Neuroscience and Biobehavioral Reviews*, *35*(3), 826–47. doi:10.1016/j.neubiorev.2010.10.003

Stoof, J. C., & Kebabian, J. W. (1981). Opposing roles for D-1 and D-2 dopamine receptors in efflux of cylclic AMP from rat neostriatum. *Nature*, *294*.

Stopper, C. M., & Floresco, S. B. (2014). What's better for me? Fundamental role for lateral habenula in promoting subjective decision biases. *Nature Neuroscience*, *17*(1), 33–5. doi:10.1038/nn.3587

Stratford, T. R., & Kelley, A. E. (1997). GABA in the nucleus accumbens shell participates in the central regulation of feeding behavior. *The Journal of Neuroscience*, *17*(11), 4434–4440.

Struthers, W. M. (2001). Sex-induced fos in the medial preoptic area: projections to the midbrain. *Neuroreport*, *12*(14), 3065–8.

Stuber, G. D., Britt, J. P., & Bonci, A. (2012). Optogenetic modulation of neural circuits that underlie reward seeking. *Biological Psychiatry*, *71*(12), 1061–7. doi:10.1016/j.biopsych.2011.11.010

Stuber, G. D., Wightman, R. M., & Carelli, R. M. (2005). Extinction of cocaine self-administration reveals functionally and temporally distinct dopaminergic signals in the nucleus accumbens. *Neuron*, *46*(4), 661–9. doi:10.1016/j.neuron.2005.04.036

Substance Abuse and Mental Health Services Administration. (2011). Results from the 2010 National Survey on Drug Use and Health : Summary of National Findings. Rockville, MD.

Surmeier, D. J., Ding, J., Day, M., Wang, Z., & Shen, W. (2007). D1 and D2 dopamine-receptor modulation of striatal glutamatergic signaling in striatal medium spiny neurons. *Trends in Neurosciences*, *30*(5), 228–35. doi:10.1016/j.tins.2007.03.008

Swaab, D. F., Chung, W. C., Kruijver, F. P., Hofman, M. A., & Ishunina, T. A. (2001). Structural and functional sex differences in the human hypothalamus. *Hormones and Behavior*, *40*(2), 93–8. doi:10.1006/hbeh.2001.1682

Swanson, L. W. (1982). The projections of the ventral tegmental area and adjacent regions: a combined fluorescent retrograde tracer and immunofluorescence study in the rat. *Brain Research Bulletin*, *9*(1-6), 321–53.

Swanson, L. W., Mogenson, G. J., & Simerly, R. B. (1987). Anatomical and electrophysiological evidence for a projection from the medial preoptic area to the "mesencephalic and subthalamic locomotor regions" in the rat. *Brain Research*, *405*, 108–122.

Sweet, W. H., & Mark, V. H. (1953). Unipolar anodal electrolytic lesions in the brain of man and cat: Report of five human cases with electrically produced bulbar or mesencephalic rractotomies. *Archives of Neurology & Psychiatry*, *70*(2), 224. doi:10.1001/archneurpsyc.1953.02320320090007

Tapert, S. F., Aarons, G. A., Sedlar, G. R., & Brown, S. A. (2001). Adolescent substance use and sexual risk-taking behavior. *The Journal of Adolescent Health*, *28*(3), 181–9.

Teneud, L. M., Baptista, T., Murzi, E., Hoebel, B. G., & Hernandez, L. (1996). Systemic and local cocaine increase extracellular serotonin in the nucleus accumbens. *Pharmacology, Biochemistry, and Behavior*, *53*(3), 747–52.

Terner, J. M., & de Wit, H. (2006). Menstrual cycle phase and responses to drugs of abuse in humans. *Drug and Alcohol Dependence*, *84*(1), 1–13. doi:10.1016/j.drugalcdep.2005.12.007

Thomas, M. J., Kalivas, P. W., & Shaham, Y. (2008). Neuroplasticity in the mesolimbic dopamine system and cocaine addiction. *British Journal of Pharmacology*, *154*(2), 327–42.

Thomas, P. (2012). Rapid steroid hormone actions initiated at the cell surface and the receptors that mediate them with an emphasis on recent progress in fish models. *General and Comparative Endocrinology*, *175*(3), 367–83. doi:10.1016/j.ygcen.2011.11.032

Thompson, T. L., & Moss, R. L. (1994). Estrogen regulation of dopamine release in the nucleus accumbens: genomic- and nongenomic-mediated effects. *Journal of Neurochemistry*, *62*(5), 1750–6.

Tobiansky, D. J., Roma, P. G., Hattori, T., Will, R. G., Nutsch, V. L., & Dominguez, J. M. (2013). The medial preoptic area modulates cocaine-induced activity in female rats. *Behavioral Neuroscience*, *127*(2), 293–302. doi:10.1037/a0031949

Tsuda, M. C., Mattei, L., Spencer-Segal, J. L., Waters, E. M., Romeo, R. D., Milner, T. A., … Ogawa, S. (2011). Estradiol acts via estrogen receptors alpha and beta on pathways important for synaptic plasticity in the mouse hippocampal formation. *Neuroscience*, *202*, 131–146. doi:10.1016/j.neuroscience.2011.11.035

Tsuneoka, Y., Maruyama, T., Yoshida, S., Nishimori, K., Kato, T., Numan, M., & Kuroda, K. O. (2013). Functional, anatomical, and neurochemical differentiation of medial preoptic area subregions in relation to maternal behavior in the mouse. *The Journal of Comparative Neurology*, *521*(7), 1633–63. doi:10.1002/cne.23251

Twining, R. C., Tuscher, J. J., Doncheck, E. M., Frick, K. M., & Mueller, D. (2013). 17b-Estradiol is necessary for extinction of cocaine seeking in female rats. *Learning & Memory*, *20*, 300–306. doi:10.1101/lm.030304.113

Tzschentke, T. M. (2007). Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. *Addiction Biology*, *12*(3-4), 227–462. doi:10.1111/j.1369-1600.2007.00070.x

Unda, R., Brann, D. W., & Mahesh, V. B. (1995). Progesterone suppression of glutamic acid decarboxylase (GAD67) mRNA levels in the preoptic area: correlation to the luteinizing hormone surge. *Neuroendocrinology*, *62*, 562–570.

Ungerstedt, U. (1971). Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. *Acta Physiologica Scandinavica. Supplementum*, *367*, 95–122.

Van Etten, M. L., Neumark, Y. D., & Anthony, J. C. (1999). Male-female differences in the earliest stages of drug involvement. *Addiction*, *94*(9), 1413–1419.

Van Swearingen, A. E. D., Sanchez, C. L., Frisbee, S. M., Williams, A., Walker, Q. D., Korach, K. S., … Swearingen, A. E. D. Van. (2013). Estradiol replacement enhances cocaine-stimulated locomotion in female C57BL/6 mice through estrogen receptor alpha. *Neuropharmacology*, *72*, 236–49. doi:10.1016/j.neuropharm.2013.04.015

Van Zessen, R., Phillips, J. L., Budygin, E. a, & Stuber, G. D. (2012). Activation of VTA GABA neurons disrupts reward consumption. *Neuron*, *73*(6), 1184–94. doi:10.1016/j.neuron.2012.02.016

Vasudevan, N., & Pfaff, D. W. (2008). Non-genomic actions of estrogens and their interaction with genomic actions in the brain. *Frontiers in Neuroendocrinology*, *29*(2), 238–57. doi:10.1016/j.yfrne.2007.08.003

Ventura, R., Cabib, S., & Puglisi-Allegra, S. (2001). Opposite genotype-dependent mesocorticolimbic dopamine response to stress. *Neuroscience*, *104*(3), 627–631.

Vernotica, E. M., Rosenblatt, J. S., & Morrell, J. I. (1999). Microinfusion of cocaine into the medial preoptic area or nucleus accumbens transiently impairs maternal behavior in the rat. *Behavioral Neuroscience*, *113*, 377–390.

Vilner, B. J., & Bowen, W. D. (2000). Modulation of cellular calcium by sigma-2 receptors : Release from intracellular stores in human SK-N-SH neuroblastoma cells. *Journal of Pharmacology and Experimental Therapeutics*, *292*(3), 900–911.

Viscarello, R. R., Ferguson, D. D., Nores, J., & Hobbins, J. C. (1992). Limb-body wall complex associated with cocaine abuse: further evidence of cocaine's teratogenicity. *Obstetrics and Gynecology*, *80*, 523–526.

Volkow, N. D., Fowler, J. S., & Wang, G.-J. (1999). Imaging studies on the role of dopamine in cocaine reinforcement and addiction in humans. *Journal of Psychopharmacology*, *13*(4), 337–345. doi:10.1177/026988119901300406

Volkow, N. D., Wang, G.-J., & Baler, R. D. (2012). Reward, dopamine and the control of food intake: implications for obesity. *Trends in Cognitive Sciences*, *15*(1), 37–46. doi:10.1016/j.tics.2010.11.001.Reward

Wagner, C. K., Eaton, M. J., Moore, K. E., & Lookingland, K. J. (1995). Efferent projections from the region of the medial zona incerta containing A13 dopaminergic neurons: a PHA-L anterograde tract-tracing study in the rat. *Brain Research*, *677*(2), 229–237. doi:10.1016/0006-8993(95)00128-D

Walker, Q. D., Cabassa, J., Kaplan, K. a, Li, S. T., Haroon, J., Spohr, H. A., & Kuhn, C. M. (2001). Sex differences in cocaine-stimulated motor behavior: disparate effects of gonadectomy. *Neuropsychopharmacology*, *25*(1), 118–30. doi:10.1016/S0893-133X(00)00248-7

Warembourg, M., & Leroy, D. (2004). Comparative distribution of estrogen receptor α and β immunoreactivities in the forebrain and the midbrain of the female guinea pig. *Brain Research*, *1002*, 55–66. doi:10.1016/j.brainres.2003.12.014

Washton, A. M., & Gold, M. S. (1984). Chronic cocaine abuse: Evidence for adverse effects on health and functioning. *Psychiatric Annals*, *10*(14), 733–743.

Weiss, R. D., Martinez-Raga, J., Griffin, M. L., Greenfield, S. F., & Hufford, C. (1997). Gender differences in cocaine dependent patients: a 6 month follow-up study. *Drug and Alcohol Dependence*, *44*(1), 35–40.

Westermeyer, J., & Boedicker, A. E. (2000). Course, severity, and treatment of substance abuse among women versus men. *American Journal of Drug and Alcohol Abuse*, *26*(4), 523–535.

Wetzel, C. H. R., Hermann, B., Behl, C., Pestel, E., Rammes, G., Zieglga, W., … Rupprecht, R. (1998). Functional antagonism of gonadal steroids at the 5-hydroxytryptamine type 3 receptor. *Molecular Endocrinology*, (October), 1441–1451.

Whishaw, I., & Kornelsen, R. (1993). Two types of motivation revealed by ibotenic acid nucleus accumbens lesions: Dissociation of food carrying and hoarding and the role of primary and incentive motivation. *Behavioural Brain Research*, *55*(2), 283–295. doi:10.1016/0166-4328(93)90123-8

White, N. M., Packard, M. G., & Hiroi, N. (1991). Place conditioning with dopamine D1 and D2 agonists injected peripherally or into nucleus accumbens. *Psychopharmacology*, *103*, 271–276. doi:10.1007/BF02244216

White, T. L., Justice, A. J. H., & de Wit, H. (2002). Differential subjective effects of D-amphetamine by gender, hormone levels and menstrual cycle phase. *Pharmacology, Biochemistry, and Behavior*, *73*(4), 729–41.

Whitney, J. F. (1986). Effect of medial preoptic lesions on sexual behavior of female rats is determined by test situation. *Behavioral Neuroscience*, *100*, 230–235. doi:10.1037/0735-7044.100.2.230

Wickens, J. R., Budd, C. S., Hyland, B. I., & Arbuthnott, G. W. (2007). Striatal contributions to reward and decision making: making sense of regional variations in a reiterated processing matrix. *Annals of the New York Academy of Sciences*, *1104*, 192–212. doi:10.1196/annals.1390.016

Wilcox, K. M., Paul, I. A., & Woolverton, W. L. (1999). Comparison between dopamine transporter affinity and self-administration potency of local anesthetics in rhesus monkeys. *European Journal of Pharmacology*, *367*(2-3), 175–181. doi:10.1016/S0014-2999(98)00967-4

Will, R. G., Hull, E. M., & Dominguez, J. M. (2014). Influences of dopamine and glutamate in the medial preoptic area on male sexual behavior. *Pharmacology, Biochemistry, and Behavior*, *121*, 115–23. doi:10.1016/j.pbb.2014.02.005

Wise, R. A. (1978). Catecholamine theories of reward: a critical review. *Brain Research*, *152*, 215–247.

Wise, R. A. (1982). Neuroleptics and operant behavior: The anhedonia hypothesis. *Behavioral and Brain Sciences*, *5*, 39. doi:10.1017/S0140525X00010372

Wise, R. A. (1996). Neurobiology of addiction. *Current Opinion in Neurobiology*, *6*(2), 243–51.

Wise, R. A. (1998). Drug-activation of brain reward pathways. *Drug and Alcohol Dependence*, *51*(1-2), 13–22. doi:10.1016/S0376-8716(98)00063-5

Wise, R. A. (2002). Brain reward circuitry : Insights from unsensed incentives. *Neuron*, *36*(October), 229–240.

Wise, R. A. (2004). Dopamine, learning and motivation. *Nature Reviews. Neuroscience*, *5*(6), 483–94. doi:10.1038/nrn1406

Wise, R. A. (2008). Dopamine and reward: the anhedonia hypothesis 30 years on. *Neurotoxicity Research*, *14*(2-3), 169–83. doi:10.1007/BF03033808

Wise, R. A. (2009). Roles for nigrostriatal-not just mesocorticolimbic-dopamine in reward and addiction. *Trends in Neurosciences*, *32*(10), 517–24.

Wise, R. A., Spindler, J., Dewit, H., & Gerber, G. J. (1978). Neuroleptic-induced "anhedonia" in rats : Pimozide blocks reward quality of food. *Science*, *201*(4352), 262–264.

Yahr, P., & Greene, S. B. (1992). Effects of unilateral hypothalamic manipulations on the sexual behaviors of rats. *Behavioral Neuroscience*, *106*(4), 698–709.

Yang, L. Y., & Clements, L. G. (2000). MPOA lesions affect female pacing of copulation in rats. *Behavioral Neuroscience*, *114*, 1191–1202. doi:10.1037/0735-7044.114.6.1191

Yokel, R. A., & Wise, R. A. (1976). Attenuation of intravenous amphetamine reinforcement by central dopamine blockade in rats. *Psychopharmacology*, *48*, 311–318.

Zahm, D. S. (2000). An integrative neuroanatomical perspective on some subcortical substrates of adaptive responding with emphasis on the nucleus accumbens. *Neuroscience & Biobehavioral Reviews*, *24*(1), 85–105. doi:10.1016/S0149-7634(99)00065-2

Zahm, D. S., Becker, M. L., Freiman, A. J., Strauch, S., Degarmo, B., Geisler, S., … Marinelli, M. (2010). Fos after single and repeated self-administration of cocaine and saline in the rat: emphasis on the Basal forebrain and recalibration of expression. *Neuropsychopharmacology*, *35*(2), 445–63. doi:10.1038/npp.2009.149

Zahm, D. S., Cheng, A. Y., Lee, T. J., Ghobadi, C. W., Schwartz, Z. M., Geisler, S., … Veh, R. W. (2011). Inputs to the midbrain dopaminergic complex in the rat, with emphasis on extended amygdala-recipient sectors. *The Journal of Comparative Neurology*, *519*(16), 3159–88. doi:10.1002/cne.22670

Zhang, D., Yang, S., Yang, C., Jin, G., & Zhen, X. (2008). Estrogen regulates responses of dopamine neurons in the ventral tegmental area to cocaine. *Psychopharmacology*, *199*(4), 625–35. doi:10.1007/s00213-008-1188-6

Zilberman, M., Tavares, H., & El-Guebaly, N. (2003). Gender similarities and differences: The prevalence and course of alcohol- and other substance-related disorders. *Journal of Addictive Diseases*, *22*(4), 61–74.